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The Intravenous Glucose Tolerance Test in Atherosclerotic Disease with Special Reference to Obesity, Hypertension, Diabetic Heredity and Cholesterol Values

By

Fredrik Wahlberg

Today patients with diabetes mellitus sooner or later develop vascular disease. In the coronary and peripheral vessels the disease is generally considered to be atherosclerotic (1 2) although this has been questioned lately (3, 4)

Clinical experience seems to show that high caloric and high fat intake has a positive influence on the degree and acceleration of vascular disease in diabetes mellitus. During the last decades th same has been postulated for atherosclerotic disease in nondiabetic individuals (5)

I diabetes mellitus the primary metabolic disturbance seems to affect carbohydrate metabolism but fat and protein metabolism are also disturbed. Disturbance of fat metabolism is generally believed to be present also in atherosclerosis and many authorities imply a causal relation between disturbed fat metabolism and atherosclerosis. Further similarities exist between adult diabetes mellitus and atherosclerotic disease in nondiabetic individuals, such as prevalence in the same agegroups, the role seemingly played by overweight, lack of exercise, composition of diet and the frequency of high cholesterol values.

For these reasons it was considered worth while to investigate carbohydrate metabolism in non-diabetic patients with atherosclerotic disease in the heart and/or the extremities. Because several of these patients also had hypertension and were obese an attempt was made to investigate the influence of these factors on the carbohydrate-metabolism. Likewise the importance of diabetic or coronary disease heredity was evaluated.

Material

I. Experimental group

The group of atherosclerotic patients consisted only of individuals with anamnestic,

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Table 1. Distribution of k-values and the mean age of the groups

	Pathological (k < 0.90)	Borderline (0.90 ≥ k ≤ 1.10)	Normal (k > 1.10)
Number of patients	44 (46%)	20 (21%)	31 (33%)
Mean group age	62 years	61 years	57 years

Methods

The intravenous glucose tolerance test was performed as follows

After duplicate fasting blood samples were obtained 25 grams of glucose in 60 per cent solution were injected intravenously The injection time was 2—4 minutes. Zero time was set at the end of the injection. Single blood samples were taken after 10, 20 25 30, 35, 40, 45, 50 and 55 minutes. Duplicate samples were taken after 60 minutes.

Capillary blood samples were used and blood sugar estimations made by glucose-specific enzymatic (glucose-oxidase) method as described by Marks and modified by S. Laurell

Approximately 20 minutes after the injection the blood glucose values form straight line when plotted in semilogarithmic system From extrapolation of this line the half-life of the glucose load is calculated. The test result is expressed as the slope of this line (k value) and is calculated $k = 0.693 \times 100/t_{\frac{1}{2}}$ where $t_{\frac{1}{2}}$ is the half life of the glucose load. Steep slopes give short half-life and high k-value flat slopes give long half-life and a low k-value. This way of evaluating the test is recommended by Hamilton and Stein (6) and Ikkos and Luft (7) See fig. 1

All the hospitalised patients were in a good nutritional state and were put on the ordinary hospital diet with no additional carbohydrate intake during the day before the test The tests were performed in the morning with the patients fasting for the last 10—12 hours.

Cholesterol values were determined by the method of S. Pearson, Stern and McGavack. Determinations were not performed in all the patients of this study but no deliberate selection was made. Arbitrarily 280 mg per cent was chosen as the upper limit of normal.

Results

The experimental group

One hundred and thirty-seven tests were performed in 95 patients. The mean age of the men was 59 and of the women 64 years.

The distribution of the age groups is shown in fig 2.

The age distribution is fairly representative of atherosclerotic patients at the Seraphimer Hospital. In a 10-year survey of the myocardial infarction material in this hospital from the years 1950—59 (8) the age-group 50—79 years constituted 84 per cent of the total. Corresponding figure in this study is 90 per cent.

According to Conard (Lundbæk (9)) Ikkos and Luft (7) and Lundbæk (9) k values lower than 0 90 are only found in diabetes mellitus and values higher than 1 10 only in individuals without diabetes. Values between 0 90 and 1 10 fall into a borderline group where patients with and without diabetes mellitus may be found In this study values lower than 0.90 have been considered pathological, values higher than 1 10 normal and values be

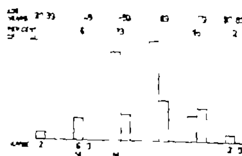
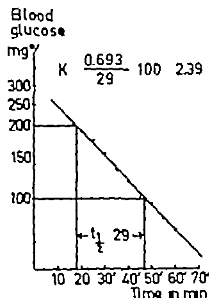


Fig. 2. Distribution of age-groups among the atherosclerotic patients



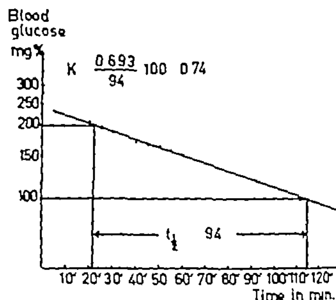


Fig. 1. Calculation of K-values.

clinical and laboratory evidence of atherosclerotic disease in the heart or extremities. None of the patients had anamnestic evidence of diabetes mellitus. In all fasting blood-sugar was repeatedly normal and glycosuria absent. None showed any signs of endocrine, hepatic or renal disease on routine clinical and laboratory investigations. No patient was severely ill at the time of the test.

Ninety-five patients were included as follows:

1. All patients with acute myocardial infarction who have been treated in the department of medicine at the Serafimer Hospital since the beginning of this investigation provided their condition permitted the test. This group consisted of 39 patients, 25 men and 14 women. Twenty-four of these were tested two or three times during the first week of hospitalisation and 4-6 weeks afterwards. Fifteen patients were tested only once 2—4 weeks after hospitalisation when they were in good condition.

2. Twenty-two patients, 19 men and 3 women with myocardial infarction from 6 months to 10 years earlier.

3. Thirteen patients, 10 men and 3 women with angina pectoris.

4. Six patients, 5 men and 1 woman, with intermittent claudication.

5. Fifteen patients, 8 men and 7 women with anamnestic, clinical and laboratory signs of atherosclerotic heart-disease but who were not included in groups 1—4.

In groups 2—5 all but 4 patients were hospitalised at the time of the test. No criteria other than atherosclerosis was used for selection. The author chose the patients from the diagnostic face-sheet in the wards without knowing anything further about them and without having seen them before.

II. *Control group*

The control group consisted of hospitalised patients without anamnestic, clinical or laboratory evidence of atherosclerotic disease or diabetes mellitus.

No patient was severely ill at the time of the test.

Forty-two patients were included, 22 men and 20 women.

Seven patients had gastro-intestinal disease, 5 patients had non-atherosclerotic heart disease, 5 patients had respiratory disease, 2 patients had hyperthyreoidism, 2 patients had venous thrombosis of the leg, 1 patient had dystrophia myotonica, 5 patients were only obese, 4 patients had fractures, 2 patients had been operated on for inguinal hernia and in 8 patients no evidence of organic disease was found.

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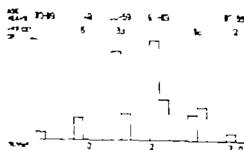


Fig. 2. Distribution of age-groups among the atherosclerotic patients.

Table II. Distribution of cholesterol values, obesity, hypertension, heredity for diabetes mellitus and coronary disease

	Number of patients	Pathological (k < 0.90)	Borderline (0.90 ≥ k ≤ 1.10)	Normal (k > 1.10)
Cholesterol < 290 mg	[illegible]	[illegible]0 (54)	[illegible] ([illegible])	9 ([illegible])
Cholesterol > 290 mg	5[illegible]	[illegible] (44)	10 ([illegible])	18 ([illegible])
Obesity > 10	[illegible]	16 (59)	8 (30)	3 (11)
Hypertension	16	5 (31)	3 (19)	8 (50)
Heredity for diabetes mellitus	10	6 (60)	1 (10)	3 ([illegible])
Heredity for coronary disease	18	6 (33)	5 (28)	[illegible] ([illegible])

tween 0.90 and 1.10 falling into a border line group.

The distribution of k values and the mean age of the groups are shown in table I.

The difference in mean-age of the groups is probably not significant.

The distribution of cholesterol values, hypertension, obesity, heredity for diabetes and coronary disease is shown in table II.

Cholesterol values, hypertension and coronary heredity showed no correlation to pathological glucose tolerance. Obesity and diabetic heredity showed a higher frequency of pathological glucose tolerance than the rest of the atherosclerotic patients.

In the myocardial infarction group alone there were 61 patients, 44 men and 17 women. The results in this group are similar to those in the total atherosclerotic group in such a way that a separate presentation is unnecessary. Only it should be mentioned that in the myocardial infarction group the mean age for those with pathological k values was [illegible] years and for those with normal k values 59 years, i.e. a smaller difference than in the total atherosclerotic group.

The distribution of pathological, borderline and normal k values among the age-groups is shown in fig. 3.

Only in the 60—69 year group there is a clear predominance of pathological values. No conclusions can be drawn that age itself predisposes for pathological k values, as will be shown further in the control group.

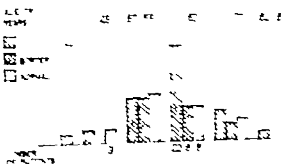


Fig. 3. Distribution of pathological, borderline and normal k values among the age-groups.

The control group

Fifty tests were performed in 4[illegible] patients.

Table III. Distribution of k values and the mean age of the groups

	Pathological k < 0.90	Borderline 0.90 < k ≤ 1.10	Normal k > 1.10
Number of patients	4 (10)	8 (19)	30 ([illegible])
Mean group age	[illegible] years	59 years	[illegible] years

Table IV. The distribution of cholesterol values, obesity and diabetic heredity in the control group

	Number of patients	Pathological (k < 0.90)	Borderline (0.90≥k≤1.10)	Normal (k > 1.10)
Cholesterol < 280 mg %	14	1 (7%)	3 (21%)	10 (72%)
Cholesterol > 280 mg %	12	2 (17%)	2 (17%)	8 (66%)
Obesity > 10 %	11	2 (18%)	2 (18%)	7 (64%)
Diabetic heredity	7	2 (29%)	0	5 (71%)

tients. The mean age of the men was 59 years and of the women 54 years.

The distribution of the age-groups is shown in fig. 4.

The age distribution of the control group does not correspond exactly to that of the experimental group. This divergence is not the result of deliberate selection of patients but of the difficulty to get suitable in-patients.

The distribution of k-values and the mean age of the groups is shown in table III.

A short presentation of the control patients with pathological k-values might be of interest.

1. An 81 years old man with a one week old fracture of the right leg and who was immobilised in bed.

2. A 53 years old woman with a calculated 23 per cent overweight and cholesterol values averaging 380 mg%.

3. A 51 years old woman with strong diabetic heredity and cholesterol values averaging 360 mg%.

4. A 41 years old man with strong diabetic heredity and a calculated 30 per cent overweight.

Four of the 8 patients in the borderline group showed approximately the same clinical abnormalities.

The distribution of cholesterol values, obesity and diabetic heredity is shown in table IV. None of the control patients had hypertension or coronary heredity.

In the control group the cholesterol values showed no correlation to the pathological k-values. Whether obesity or diabetic heredity are correlated to pathological k-values is uncertain. The results seem to be in contrast to those of the atherosclerotic group.

Variations of the k-values in the same patient on repeated test

Forty-three patients were tested two or three times. Nineteen showed lower k-values and 24 showed higher k-values on the second test. It is thus obvious that there was no difference between the first and second test. Thirty-six patients belonged to the same classification group (pathological, borderline, normal) on repeated tests. The mean difference between the k-values of the same patient was 0.14. This is thought remarkable as the general condition of the patient in most cases was different at the time of the first and second test.

In 5 patients the k-values fell into adjoining groups on repeated test. In 3 of them the difference in k-values was 0.12,

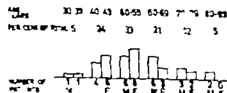


Fig. 4. Distribution of age-groups in the control group.

0.16 and 0.19 the values being on different sides of the chosen limiting values of the groups. In 2 patients the difference was 0.25 and 0.30.

Two patients had k values in the pathological and normal group on repeated test. One was a 78 years old woman in the control group with fever of unknown origin. The first test was performed the day after the patient's admission to the hospital and the k value was 0.85. Next test 2 weeks later when the patient was almost well gave a very uneven curve with an estimated k value around 1.60. The third test 4 weeks later when the patient was absolutely well and ambulatory gave a k value of 1.14.

The other was a 53 years old male who was overweight and had his first myocardial infarction. The initial test was performed 4 days after admission and gave a k value of 0.76. The second test 5 weeks later gave a k value of 1.33. The third test 6 months after the first test gave a k-value of 0.95.

Discussion

This study has shown that in a group of unselected patients with atherosclerotic disease and without known diabetes mellitus the intravenous glucose tolerance test was pathologic in 46 per cent of the patients. In only 33 per cent the test was normal. Obesity and diabetic heredity seemed to predispose to pathologic glucose tolerance whereas hypertension, hypercholesterolemia and coronary disease heredity did not. Age itself was apparently without influence but further study is needed.

In the control group 10 per cent had pathological glucose tolerance and 71 per cent were normal. In the control group it is uncertain whether obesity and diabetic heredity predisposes to pathologic glucose tolerance. The number of patients is small but the results seem to be in contrast to those of the atherosclerotic patients. Cholesterol values and age were of no influence. No patient with hypertension or coronary heredity happened to be included in the control group.

The composition of the control group might be criticized as it does not consist of healthy individuals. Included were patients in age groups in which atherosclerosis must exist frequently although not detected clinically. Also several patients were included who had the combination of obesity, diabetic heredity and hypercholesterolemia and such patients are known to show a high frequency of clinical diabetes mellitus.

The object of this study was to investigate the frequency of pathologic glucose tolerance in patients with atherosclerotic disease. It was thought that to evaluate the results properly the control group should consist of patients without atherosclerotic disease but with as many as possible of the features complicating the atherosclerotic patients and living under the same conditions at the time of the test.

The significance of the decreased glucose tolerance in the patients of this study is not known at present. Factors known to decrease glucose tolerance such as poor nutritional state, hemochromatosis, endocrine disorders (Cushing's disease, acromegaly), wasting or malignant neoplastic disease were absent. Although no special attention was paid to the composition of the food, this factor cannot have been of importance since both the experimental and the control group were approximately on the same diet. Under these premises decreased glucose tolerance has only been seen in diabetes mellitus.

Diabetes mellitus represents a complex metabolic disturbance characterized by

decreased glucose tolerance. By definition then 46 per cent of the patients with atherosclerotic disease in this study have subclinical diabetes mellitus compared to 10 per cent of the control group. It should be mentioned that in the studies on myocardial infarction from Malmö by Biörck et al. (10) the prevalence of clinical diabetes mellitus was about five times higher in infarct patients than in the population. The frequency ratios were about the same in all age groups in both sexes.

The etiology and pathogenesis of diabetes mellitus and atherosclerosis are unknown today. It is generally accepted that all patients with diabetes mellitus sooner or later develop atherosclerotic disease. Whether the reason for this primarily is the disturbed carbohydrate metabolism or the disturbed fat metabolism or some other factor is not known. It may be possible that subclinical diabetes mellitus predisposes for vascular disease in the same way as clinical diabetes mellitus. If so the result of this preliminary investigation raises the question if the primary disease of the atherosclerotic patients with pathologic glucose tolerance is subclinical diabetes mellitus.

Further study will show whether the patients of this study with pathologic glucose tolerance will develop clinical diabetes mellitus more frequently than the patients with normal glucose tolerance. It will also be shown whether the patients with pathologic glucose tolerance will have a worse prognosis and if so, whether this prognosis can be changed. During the short observation time of this study (6 months) 4 of the patients with atherosclerotic disease have died, all of them having had pathologic glucose tolerance. At autopsy all showed advanced general atherosclerosis. No other patient in this study has died yet.

Summary

The result of this preliminary investigation shows that in patients with atherosclerotic disease but without clinical diabetes mellitus the glucose tolerance was pathologic in 46 per cent and normal in 33 per cent. Corresponding figures in the control group were 10 per cent and 71 per cent. The result cannot be explained by prevalence of obesity, diabetic heredity, hypercholesterolemia or old age. The significance and implications of these findings are briefly discussed.

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From the Department of Clinical Physiology the Third Outpatient Department of Internal Medicine and the Second Department of Anaesthesiology Sahlgrenska sjukhuset, University of Göteborg, Sweden

The Effect of Stellate Ganglion Block on the Relationship between Extra and Intra-arterially Recorded Brachial Pulse Waves in Man

By

F Heyman and K. Stenberg

Previous studies have shown that the pulse wave recorded from outside the artery leads the pressure wave in phase and has steeper gradients (1 2) The dif ference may be assigned to variations in distensibility (tone) of the arterial wall during each pulse cycle. The atrial systolic waves in the peripheral pulse may also be attributed to variations in tone (3 4) They were eliminated by nerve block, a sign that they are due to a reflex (4) It may be supposed that the d fference between extra- and intra-arterial records of pulse waves is due to a similar reflex and thus may also be eliminated by nerve block.

Methods

A piezo-electric microphone was used for recording the brachial pulse extra-arterially while the intra-arterial pressure pulse from pproximately the same point of the artery was recorded through polyethylene catheter attached to strain gauge manometer The curves were recorded simultaneously with an electrocardiogram by a direct writing electrocardiograph. For details of the methods see previous papers (1 2, 4)

Stellate ganglion block was performed by the anterior approach (5) using 15 ml of a 1.0 per cent solution of xylocaine without adrenaline (4) or in a few experiments 15 ml of a 1.0 per cent solution of carbocaine (4)

Pulse curves were recorded at short intervals during one hour before and one to tw hours after the block, using the paper speeds 5 10 and 100 cm per second.

Material

Ten men were examined three of them were healthy (22, 25 and 75 years of age) seven were untreated patients with benign hypertension from the out-patient department (ages ranging between 31 and 71 years and blood pressure between 178/97 and 261/122 mm Hg as measured intra-arterially prior to the blocking).

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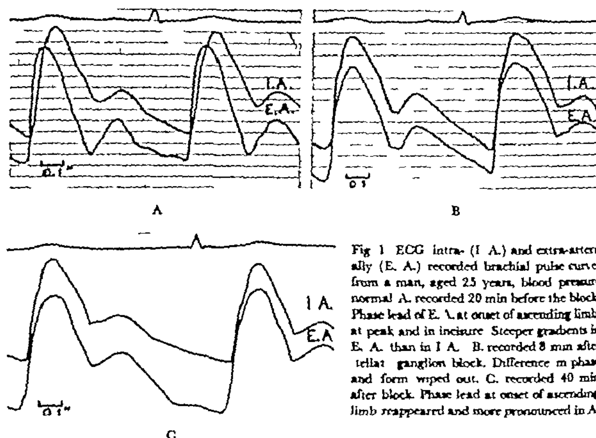


Fig 1 ECG intra- (I A.) and extra-arterially (E. A.) recorded brachial pulse curves from a man, aged 25 years, blood pressure normal A. recorded 20 min before the block. Phase lead of E. A. at onset of ascending limb, at peak and in incisure Steeper gradients in E. A. than in I A. B. recorded 8 min after stellate ganglion block. Difference in phase and form wiped out. C. recorded 40 min after block. Phase lead at onset of ascending limb reappeared and more pronounced in A.

Results

In eight of the ten cases examined the relationship between the extra and intra arterially recorded pulse curves changed in connection with the stellate ganglion block while no change was seen in two cases. The principle phenomena were

1 The phase lead of the extra arterial over the intra arterial curve at the beginning of the ascending limb decreased or disappeared (fig 1 A and B)

2 The difference in form of the two curves disappeared mainly through modification of the extra arterial curve, the gradients of which became more sloping and the peak more rounded and thus congruent with the intra arterial curve (fig 1 A and B)

3 The congruency in form resulted in disappearance of the phase lead of the extra arterial curve at the peak and in the incisure of the pulse wave (fig 1 A and B)

In some cases of hypertension the summits of the pulse waves were plateau formed or rounded and of different shapes in the two curves (the phase relationship between the curves was thus difficult to assess) In these cases the difference in form decreased or disappeared after the block, mainly through transformation of the extra arterial curve.

The phenomena described above were seen a few minutes after the block. They subsided gradually beginning with reappearance of the phase lead of the extra arterial curve at the onset of the ascending limb Sometimes an increase of this lead as compared to the one seen before the blocking was noticed at this time (fig 1 C) The difference in the shapes of the curves reappeared more slowly

The pulse frequency and blood pressure were as a rule little affected by the block.

Discussion

The present study shows that the difference between extra- and intra-arterial records of pulse waves may be eradicated by stellate ganglion block. This confirms that the pulse curve recorded from outside the artery is dependent not only on the inner pressure but also on some other factor.

According to Womersley and McDonald (6) the radial dilatation of an artery is related to the velocity of flow and not directly to the pressure. As discussed in a previous paper (7) the difference between extra- and intra-arterially recorded pulse waves cannot be explained in this way. It may be added in this connection that the flow and thus the velocity of flow in the brachial artery is probably increased by stellate ganglion block. This should increase the lead of the extra-arterial curve if this explanation is correct. The opposite has been shown to be the case here.

According to Heyman (1, 2, 4, 7) nervous impulses influence the tone of the arterial wall rhythmically with each heart beat, resulting in (1) the difference between extra- and intra-arterially recorded pulse waves (1, 2), (2) the atrial systolic wave in the peripheral pulse (3, 4), (3) pulse synchronous movements of the arterial wall peripheral to an obstruction in the circulation (8).

The atrial waves were supposed to be due to a reflex involving baroreceptors in the atria and roots of the large veins with their afferent fibres and, as efferent pathways, the sympathetic fibres to the smooth muscles of the artery (4). Block of the efferent path eliminated the atrial waves (4). One may assume that there is a similar reflex induced by pressure wave stimulation of the baroreceptor areas on the arterial side (4). Block of the efferent path (stellate ganglion block) should extinguish the variations in tone due to this reflex and thus eradicate the difference between extra- and intra-arterially recorded pulse waves as we have seen here. The result may thus be taken as evidence for reflex interference with the pulse wave and is well in line with the studies referred to above (1, 2, 4, 8).

Summary

The effect of stellate ganglion block on the relationship between extra- and intra-arterially recorded brachial pulse waves in man was examined in ten cases. In eight of them the difference in phase and form seen before the block was reduced or eradicated by the block.

The result is taken as evidence for reflex interference with the pulse wave.

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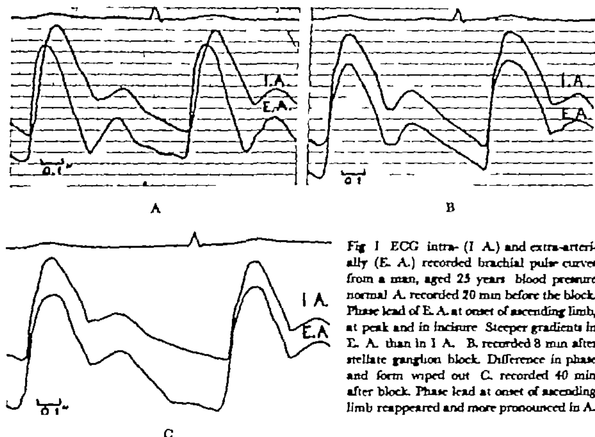


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Results

In eight of the ten cases examined the relationship between the extra and intra arterially recorded pulse curves changed in connection with the stellate ganglion block while no change was seen in two cases The principle phenomena were

1 The phase lead of the extra arterial over the intra arterial curve at the beginning of the ascending limb decreased or disappeared (fig 1 A and B)

2 The difference in form of the two curves disappeared mainly through modification of the extra arterial curve the gradients of which became more sloping and the peak more rounded and thus congruent with the intra-arterial curve (fig 1 A and B)

3 The congruency in form resulted in disappearance of the phase lead of the extra-arterial curve at the peak and in the incisure of the pulse wave (fig 1 A and B)

In some cases of hypertension the summits of the pulse waves were plateau formed or rounded and of different shapes in the two curves (the phase relationship between the curves was thus difficult to assess) In these cases the difference in form decreased or disappeared after the block, mainly through transformation of the extra arterial curve.

The phenomena described above were seen a few minutes after the block They subsided gradually beginning with reappearance of the phase lead of the extra arterial curve at the onset of the ascending limb Sometimes an increase of this lead as compared to the one seen before the blocking was noticed at this time (fig 1 C) The difference in the shapes of the curves reappeared more slowly

The pulse frequency and blood pressure were as a rule little affected by the block

Discussion

The present study shows that the difference between extra- and intra-arterial records of pulse waves may be eradicated by stellate ganglion block. This confirms that the pulse curve recorded from outside the artery is dependent not only on the inner pressure but also on some other factor.

According to Womersley and McDonald (6) the radial dilatation of an artery is related to the velocity of flow and not directly to the pressure. As discussed in a previous paper (7) the difference between extra- and intra-arterially recorded pulse waves cannot be explained in this way. It may be added in this connection that the flow and thus the velocity of flow in the brachial artery is probably increased by stellate ganglion block. This should increase the lead of the extra-arterial curve if this explanation is correct. The opposite has been shown to be the case here.

According to Heyman (1, 2, 4, 7) nervous impulses influence the tone of the arterial wall rhythmically with each heart beat, resulting in (1) the difference between extra- and intra-arterially recorded pulse waves (1, 2), (2) the atrial systolic wave in the peripheral pulse (3, 4), (3) pulse synchronous movements of the arterial wall peripheral to an obstruction in the circulation (8).

The atrial waves were supposed to be due to a reflex involving baroreceptors in the atria and roots of the large veins with their afferent fibres and, as efferent pathway, the sympathetic fibres to the smooth muscles of the artery (4). Block of the efferent path eliminated the atrial waves (4). One may assume that there is a similar reflex induced by pressure wave stimulation of the baroreceptor areas on the arterial side (4). Block of the efferent path (stellate ganglion block) should extinguish the variations in tone due to this reflex and thus eradicate the difference between extra- and intra-arterially recorded pulse waves as we have seen here. The result may thus be taken as evidence for reflex interference with the pulse wave and is well in line with the studies referred to above (1, 2, 4, 8).

Summary

The effect of stellate ganglion block on the relationship between extra- and intra-arterially recorded brachial pulse waves in man was examined in ten cases. In eight of them the difference in phase and form seen before the block was reduced or eradicated by the block.

The result is taken as evidence for reflex interference with the pulse wave.

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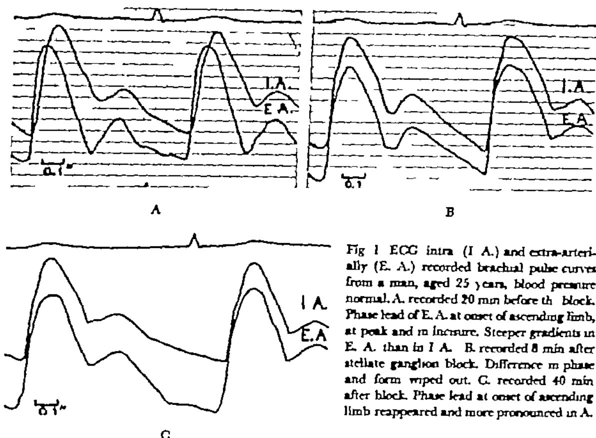


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Acta Medica Scandinavica. Vol. 171 fasc. 1, 1962

From Kronprinsessan Lovisas Hospital for Sick Children, Stockholm, the Psychiatric Department of the University of Uppsala at Ulleråkers Hospital, Ulleråker and the Institute of Histology and Paediatric Department, University of Gothenburg, Sweden

An X-linked, Recessively Inherited Syndrome Characterized by Grave Mental Deficiency, Epilepsy, and Endocrine Disorder

By

Mats Börjeson, Hans Forssman and Orla Lehmann

We have encountered three extremely feebleminded men, all of a peculiarly grotesque appearance. Two of them were half-brothers living in the same institution and the third was in another institution far away. This man looked so much like the other two that we suspected they were related even before we had found out that he was a half-brother of the mother of the two others. We have not been able to find any description of this defect anywhere in the literature.

For various reasons it was not possible to examine all three to the same extent. One of them died shortly after we had begun our study. They were all extremely backward and inclined to get agitated, making some kinds of examination very difficult. The main features of the syndrome in these men were grave mental deficiency with I. Q. s between 20 and 30, hypogonadism, obesity, a grotesque face with fatty swelling of the soft tissues and large ears. All three were dwarfs. Two of them suffered from epilepsy.

We gathered as much information as we could about the family of these men, though the relatives did not like our probing into their family. Their pedigree is shown in fig. 1. As seen there, three of the females in the family were moderately feebleminded. There was an unusually large number of illegitimate births in every generation.

The following gives the data we were able to obtain about different members of the family.

I 1. Female, 1867—1938, probably legitimate, but herself the mother of six illegitimate children. No record of mental deficiency. Worked as a domestic servant in a parsonage.

II 1. Female, 1890—91, died 7 months old from unspecified cause.

Submitted for publication June 28, 1961

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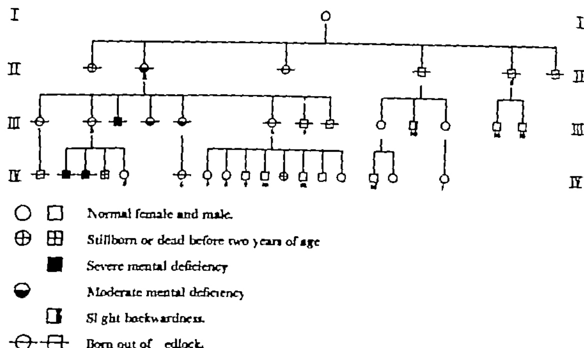


Fig 1 Pedigree.

II 2 Female, 1892— Had eight illegitimate children the last four probably by the same man Judging by conversation with her in 1961 she had an I Q between 50 and 60 As seen from fig 2 she had narrow slits between the eyelids and fairly large ears She was of short stature (148 cm) and had slight kyphosis. She had the dry skin typical of hypometabolism. It was not possible to make a thorough clinical examination She was known for her bad temper and she had never been able to keep a job long

II 3 Female, 1894— This woman was traced through the documents of nine parish registers up until 1960 with nowhere any mention of mental deficiency She earned her own living and apparently had a good income at any rate she had not received any public support during the last six years. There was no reason to suspect any mental or other defect She was unmarried

II 4 Male, 1897— No reason to suspect mental defect. He had worked as a farm hand and real estate agent, but was now prematurely pensioned becaused of cardiosclerosis. We have read his medical records

II 5 Male 1901— Foreman, of apparently normal intelligence

II 6 Male, 1905—18, killed in threshing accident. No record of mental defect in parish register Study of the records at his school showed that he had above average marks.

III 1 Female 1915— Unmarried housekeeper with one illegitimate son The district clergyman who knew her well said that she was of definitely normal intelligence.

III 2 Female, 1918— This woman refused to talk to us but, according to the guardian appointed for her children she was of normal intelligence and ordinary appearance She had two illegitimate sons both of whom had the defect in question and two legitimate children one stillborn

III 3 Male 1923— One of the probands (fig 3—4)


Fig 2. II 2 in pedigree.


Fig. 3 Proband III 3, at the age of 32

III 4 Female, 1925— After going to a school for the feebleminded, she was transferred in 1938 to a workshop for feebleminded. In 1941 and 1953 her I Q (Terman-Merrill) was 70 She was examined by us in 1957 we found no abnormality in her appearance and no signs of endocrine disorder She was discharged from the workshop in 1957 and has since earned her own living

III 5 Female, 1926— This woman had not been able to keep up in an ordinary school and had left when she was 12. She tried being a domestic servant but could not manage any of her jobs. She had an illegitimate child when she was 22 She was put in an institution for feebleminded. After that she gradually became aggressive and finally obviously psychotic with auditory hallucinations, and she was transferred to a hospital for sick feebleminded, where she still is. Intelligence testing showed an I Q of 63 (Terman-Merrill) Examination with a Swedish Point Scale test (Wåhlén) in 1954 showed an intelligence age of about 8. When we examined her in 1955, we found her to be well built (fig 5) with no physical abnormality or sign of endocrine disorder

III 6 Female, 1927— Farmer's wife, of normal intelligence according to the district schoolteacher and nurse.

III 7 Male, 1929— Earns his own living no reason to suspect mental deficiency

III 8. Male, 1931— Earns his own living no reason to suspect mental deficiency

III 9 Female, 1927— Janitor's wife. Hospitalized a short while in 1955 for cardiac palpitation. We read her hospital records. No reason to assume that she was mentally deficient

III 10 Male, 1930— Treated at an orthopedic hospital for severe rachitic



Fig 4 Proband III 3 at the age of 32



Fig 5 III.5 in pedigree

kyphoscoliosis. Perusal of his hospital records revealed that he began to walk when he was one and that he was slightly backward and had to be taught in a special class. There was no mention of any physical abnormality aside from his rachitic hump He was said to be thin.

III 11 Woman 1933— Treated at the Psychiatric Department of the University of Uppsala for obsessions during lactation She was intelligent had gone through business school and had worked in the office of an insurance company for five years. There was no note of any abnormality in her appearance

III 12 Male, 1928— Taxidriver No reason to suspect mental deficiency

III 13 Male, 1930— Seaman No reason to suspect mental deficiency

IV 1 Male 1944— Went to ordinary school and considered intelligent.

IV 2 Male, 1940—60 One of the probands, (fig 6—7)

IV 3 Male, 1943— One of the probands, (fig 8—9)

IV 4 Male 1954 stillborn Autopsy showed a full term child with no external or internal signs of abnormality Death was caused by premature detachment of the placenta.



Fig 6. Proband IV 2, at the age of 18.

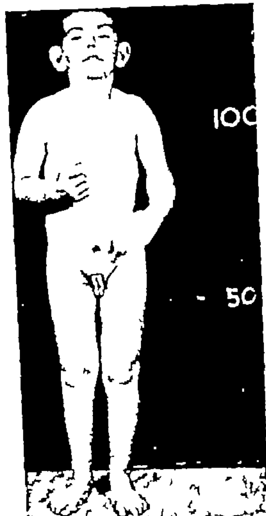


Fig 7 Proband IV 2 at the age of 18.

IV 5. Female, 1935— According to her appointed guardian, she was healthy and normally developed, and ordinary looking in every respect.

IV 6 Female, 1949— Went to ordinary school, not backward or abnormal in any respect.

IV 7—10 and IV 12. All normal according to teachers and all of ordinary appearance. They were born in 1946 1947 1948 1949 and 1952, respectively

IV 11 Female, 1950—51 Died of acute bronchopneumonia. No malformation or anything else of note recorded in hospital documents. No autopsy

IV 13—14 born in 1954 and 1959 According to the district nurse these children were of ordinary intelligence and appearance.

IV 15 Male, 1951— This boy was of normal intelligence and appearance according to the Child Welfare Officer and school nurse

IV 16 Female 1937— of normal intelligence and appearance according to Child Welfare Bureau.



Fig 8. Proband IV 3, at the age of 16.

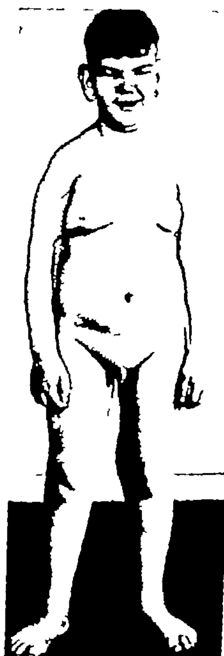


Fig 9 Proband IV 3, at the age of 16

IV 17 Female, 1958— of normal intelligence and appearance according to Child Welfare Bureau

Clinical description of the three probands

III 3. Male, 1923— (fig 3—4) illegitimate, father unknown. According to a hospital record from 1930 he began to walk when he was 4 When he was 4 he also had one to three epileptic fits a day but it was not known when he first began having them. He never learned to speak properly He was extremely aggressive from the very beginning He had been institutionalized since he was very young and had been in four homes for the feeble minded When he was 27 he was admitted to a hospital for the hard to-manage severely feebleminded where he still was. We examined him 1955 and 1960 the photographs were taken n 1955 when he was 32 He had an I Q of about 20 He was restless, hyperactive and often highly aggressive when he would bite and claw the people around him. He still had epileptic fits. He showed no other neurologic abnormality such as paralysis or spasticity

His grotesque appearance is obvious from the photographs. The skin on his face and the subcutaneous tissue on his forehead, cheeks

and eyes were swollen. His palpebral fissures were so narrow that he had to tilt his head back to be able to find his way about, and sometimes he had to hold them open with his fingers. His ears were enormous and swollen but not deformed. The panniculus adiposus was abnormally increased all over his trunk, but especially in the mammary region. He was 149 cm tall. He was extremely knock-kneed. There was a large space between his first and and second toes. *His genital status in 1955* when he was 32, is seen from fig 4 He had no terminal hair and a tiny penis and no testicles could be palpated. In 1960, a trace of sexual hair had appeared, his penis had grown a little, though it was still tiny and soft testicles the size of haricots could be palpated in the scrotum. *He had been given thyroid compounds* during the intervening years, but no other hormones. The prostate, only examined in 1960, was tiny and showed a suggestion of a middle furrow

The patient' heart and lungs were normal. The thyroid gland was of normal size and consistency He appeared to see and hear normally except that the hypertrophy of the soft tissues restricted his field of vision.

Laboratory data

Three cultures of bone marrow showed a normal number of chromosomes and a normal *idiogram in 3 mitoses.*

Nothing abnormal was noted in the amino acid pattern of the urine.

The *serum cholesterol*, measured three times between 1956 and 1958 varied between 230 and 260 mg per 100 ml.

P. B. I. was 8.6 µg per 100 ml of serum.

IV 2. Male 1940—60, Illegitimate, of known paternity The father had had skilled occupational *training and must have been* intelligent w did not learn anything else about him.

The patient had spent all his life in institutions, mostly in homes for the feebleminded. He was extremely deficient mentally and never learned to speak. H could walk when he was 20, but it was uncertain when he had learned to do so. It was impossible to give him any psychometric tests. He was sometimes extremely aggressive and bit and scratched the people *around him. H* wet and soiled his clothes His records show that he had had t *least one distinct epileptic* attack. He showed no other signs of neurologic abnormality such as paralysis or spasticity

Fig. 6—7 taken when the patient was 18, show how he looked. When these photographs were taken, he had been ill and had lost weight. His face was deformed by fatty swelling of the subcutaneous tissue, especially on the cheeks and around the eyes. The palpebral fissures were very narrow His ears were enormous but *not misshaped. Examination* when he was 18 revealed an bnormal amount of subcutaneous fat especially in the mammary region. When he was 20 he was 136 cm tall. He showed a suggestion of knock-knees and a large space between his first and second toes. At the age of 20 he showed no signs of puberty — no terminal hair infantile external genitals and soft pea sized testicles in the scrotum. (The utopsy gave the prostate as normal, but this must have meant apart from its size.) The patient appeared to have seen and heard normally Nothing was noted about the thyroid gland.

The autopsy was done routinely and not after reference from us. Broncho-pneumonia was given as the cause of death. No abnormality was noted in the internal organs. The pancreas and kidney were said to be normal. A mixed concrement the size of a nut kernel was found in the gall-bladder N gross deformity or lesion was seen in the brain. Microscopic study revealed abnormally few ganglion cells in the cortex, and several with pyknotic nuclei. Gliosis was observed both in the cortex and white matter

Laboratory data

Hormone analysis revealed less than 13 mouse units of t tal gonadotropin, 1 mg of 17-ketosteroids, and 2.8 mg of 17-hydroxy-corticosteroids per 24-hour output of urine.

II 3. Male, 1943— Half-brother of foregoing with same mother H was born out of wedlock his father who was known, did skilled work and may be assumed to be of normal intelligence but w know nothing else about him. The patient was sent to a children hospital when he was an infant and had lived at a home for feebleminded since he was one. He could walk and talk, but it is not known when he learned these bilities. He had a sticky nature H could be kept busy to some extent. His I Q (Terman-Merrill) amounted to 28



Fig. 8. Proband IV .3 at the age of 16

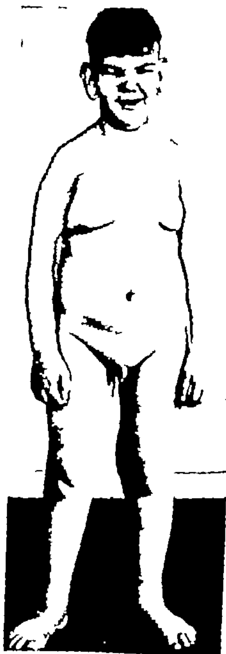


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in 1959 and his S. Q (Vineland) to 37 in 1956. He had not had any epileptic fits and no motor disorders of central origin. Fig 8—9 show how he looked when he was 16. His face exhibits fatty swelling of the subcutaneous tissue, particularly on his cheeks and around his eyes. He had extremely narrow palpebral fissures and his ears were large but not deformed He was obese all over with a large accumulation of fat in the breast region. He was 151 cm tall He was knock kneed and showed a wide space between his first and second toes. He showed no signs of sexual maturation at the age of 16, — no terminal hair a small penis and impalpable testicles.

Laboratory data

Analysis of the blood revealed normal conditions, including a differential count of the white cells.

The serum sodium amounted to 154 mEq and the potassium to 5 mEq per litre The basal metabolic rate was 24 per cent. Tracer iodine examination showed a low uptake and high excretion. P B I amounted to 5 6 μg per 100 ml serum.

A double glucose tolerance test revealed normal conditions.

The total gonadotropins amounted to less than 6.5 mouse units per 24-hour urinary output, which is low but not definitely abnormal The 17 ketosteroids and 17 hydroxycorticosteroids lay at a low but not definitely abnormal level.

Microscopic examination of a testicular specimen showed prepubertal testicular tissue but virtual aplasia of the germinal cells and interstitial fibrosis.

A negative sex-chromatin pattern was found on examination of a specimen of skin

A normal chromosome count was found on tissue culture of the bone marrow

On roentgenography of the skull it was seen that the supraorbital region projected an unusual amount and was abnormally large, but there was nothing else of note

Moderate generalized slow dysrhythmia was seen in the electroencephalogram but no local or paroxysmal disorder

Nothing abnormal was noted in the amino acid pattern of the urine.

Serum lipids Cholesterol 180 mg/100 ml, phospholipids 190 mg/100 ml (sphingomyelins 49 mg/100 ml) triglycerides 94 mg/100 ml. The investigation thus showed normal values for serum lipids.

Examination of the eyes showed normal media and eyegrounds, and hyperopia of 3 diopters.

Discussion

Clinical analysis

The syndrome involves a lesion on the cerebral cortex the idiocy and epilepsy are clinical proof of this and anatomical proof was forthcoming from the autopsy in one case. It also involves a severe endocrine disorder consisting of hypogonadism, dwarfism and hypometabolism the last two may either be a sign of hypogonadism or be independent disorders. There is reason to assume that the endocrine disorder stemmed from the hypophyseal diencephalic region.

Genetic analysis

The syndrome occurred in three men in the family and in practically the same form in all three (The only major difference was that one of the men had not yet showed signs of epilepsy at the age of 17) None of the females in the family had the syndrome On the other hand, moderate mental deficiency was noted in three women two of them half sisters of one of the affected males and one of the mother of the same man The last woman showed a suggestion of some of the physical abnormalities found in the probands.

With only three persons affected in a family one has to be careful about conclusions on the pattern of heredity The large number of illegitime births in this family is a help however It seems highly improbable that it could be a question of an autosomal recessive property As it is evident that it is an extremely rare defect, it is unlikely that the same female hetero-

zygote (III:2) would meet two heterozygotic carriers of the same defect in the two men who one after the other fathered the two illegitimate probands. (That the boys had separate fathers is established, and the two men were not related.) This hypothesis would also mean that her mother (II:2) would have conceived a child with a man having the same heterozygotic state.

Conceivably it could have been an autosomal dominant gene with only a slight probability of manifestation. This possibility cannot be excluded in view of the limited amount of material, but judging from the appearance of the family tree, it does not seem as likely as the hypothesis of a sex-linked recessive gene

In our opinion, the evidence points most strongly to a sex-linked recessive gene. If such is the case the females II:2 and III:2 would be carriers of the gene. The older one of these two was moderately feebleminded and showed traces of the physical characteristics of the syndrome. Two half-sisters of III:2, i. e. III:4 and III:5 were moderately feebleminded both of these could have been heterozygotes according to this hypothesis, but neither can be proven to be so.

The possibility remains that the slight feeblemindedness in these three women was caused by heterozygosity of the gene operating in the severely defective men. The expressivity not seldom varies to such an extent i heterozygotes that some of the gene-carriers show only a trace of the defect and others no signs at all. We only point to the possibility that the feeble mindedness of the three women may be an intermediate gene effect in heterozygotes. However their feeblemindedness was nonspecific and as they lacked all other signs of the syndrome, except for II:2 a slight physical abnormalities, nothing definite can be concluded in this respect. It is quite possible that their mental retardation was of the common variety and polygenetically determined. This idea is supported by the great number of illegitimate births in the family

Summary

A four-generation pedigree including 21 persons is presented. A particular syndrome was observed in two half-brothers and a half-brother of their mother

The main features of the syndrome were: severe mental defect; epilepsy in 2 of the 3 hypogonadism, hypometabolism and dwarfism, marked obesity and swelling of the subcutaneous tissue in the face very narrow palpebral fissures; extremely large, though not deformed ears.

Three women in the family showed only moderate mental retardation.

The syndrome was considered to be due to an X-linked recessive gene, the afflicted males showing the full-blown syndrome and some of the women perhaps an intermediate gene effect.

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Further Studies on the Interrelationship between Hemosiderin and Sideroblasts in Bone Marrow Smears

By

Alexander Weinfeld and Harald A. Hansen

Rath & Finch (1) drew attention to depletion of iron stores as the initial phase in the development of iron deficiency. Since then the estimation of tissue hemosiderin in bone marrow smears has been shown a useful tool in clinical work (2, 3, 4, 5). Accordingly when the bone marrow specimen from a patient with chronic anemia contains a normal amount of hemosiderin, iron deficiency can be ruled out. However when no hemosiderin is found one cannot with certainty conclude that the iron stores are completely exhausted. Some available iron may be present in the form of ferritin which is not detectable by light microscopy and thus it is not proved that iron deficiency is the cause of the anemia.

At physiological levels of tissue iron Shoden et al. (6) found a preponderance of ferritin iron over hemosiderin iron and in normal conditions some degree of ferritin saturation is probably needed before hemosiderin deposits appear (7). It was previously supposed that iron still available for erythropoiesis but not visible as hemosiderin would be disclosed by sideroblast counting (5). The simultaneous examination of hemosiderin and sideroblasts was therefore recommended. However discrepancies between hemosiderin estimations and sideroblast counts were observed in a variety of clinical conditions. These discrepancies have previously been discussed, and an attempt made to interpret the differences in the methods. An assumption was made, that the sideroblasts represent the most readily available and more labile fraction of the storage iron pool while hemosiderin represents the deposited body iron that is somewhat less quickly utilizable. This could explain all the divergences between the results obtained by hemosiderin estimations and those from sideroblast counting (5). The present investigation was designed to test the validity of the above assumption and to provide more material for discussion of the discrepancies between hemosiderin and sideroblasts in various clinical states.

Submitted for publication June 30, 1961

during and after interruption of oral iron therapy and after parenteral iron administration

Iron withdrawn				Parenteral iron therapy					
Days	Hb g %	Sidero-blast %	Hemo-siderin 0–+++	Days	Total mg Fe given	Days	Hb g %	Sidero blast %	Hemo-siderin 0–+++
7	11.1	14	0						
5	12.7	3	0						
6	10.2	0	0						
6		2	0						
7	12.1	2	0						
6	10.8	9	0	13	2,000 i.m.	6	11.5	84	++
8		14	0	36	1,700 i.m.	130	12.7	27	–
				4	1,000 i.m.	1	9.2	60	+
				57	2,500	1	12.[illegible]	33	++
				8	2,200 i.m.	1	9.3	74	–
				52	1,500	1	12.1	90	+++
				4	1,000 i.m.	17	8.9	15	+
				4	750 i.m.	21	9.3	23	++
				4	400	1	7.8	55	+
				6	1,500 i.m.	8	11.5	60	+
				4	400	1	9.5	60	–
				21	1,500 i.m.	21	12.3	62	+
				4	1,000 i.m.	1	7.8	78	0
				5	750 i.m.	1	7.1	15	+
				6	600 i.m.	1	8.7	40	+++

the bone marrow smears now showed a normal sideroblast count in all but one of the patients, they all still lacked histochemically demonstrable hemosiderin (table I). In six of these patients the sideroblast count became normal when treated with iron for only 2 to 7 days. Patient G. N. did not show any rise of the sideroblast count after six days of oral iron therapy. He had a severe iron-deficiency anemia with a markedly accelerated hemoglobin production when put on iron therapy. This suggests that the iron administered was rapidly utilized. This may explain why no sideroblasts appeared during the first days of oral iron therapy.

b) *Withdrawal of short-term oral iron therapy.* In 5 of the patients in the preceding group a third sternal marrow aspirate was examined when the oral iron therapy had been withdrawn for 5 to 7 days. Table I shows that the sideroblast percentage again declined to the levels seen in untreated iron deficiency anemia. From 2 other patients, B. W. and E. D., bone marrow was aspirated twice only

Table I The behavior of hemosiderin and sideroblasts in cases of chronic iron-deficiency anemia, before

Before therapy					On oral iron theraphy			
Subject	Hb g	Serum iron µg	Sidero-blast %	Hemo-siderin 0—+++	Days	Hb g	Sidero-blast %	Hemo-siderin 0—+++
S. V.	10.6	81	1	0	4	10.8	35	0
V. M	9.2		8	0	10	11.5	70	0
E. M	7.–	33	5	0	11	10.2	40	0
K. B.	7 7	4	4	0	11	11 7	54	0
E. D	7 7	42	10	0	70			
S. P	8.4	45	0	0	14	9 4	67	0
B. W	7.9	42	0	0	24	10.2		
O O	11 4	53	0	0	2	11.8	0	0
V. B	9.2	25	0	0	5	10.2	44	0
D A.	9.5	108	0	0	6	10 6	64	0
D V.	5 9	78	0	0	7	10.5	37	0
I K	10.5	48	3	0	21	12 I	70	0
G N	3.3	48	0	0	6	5.2	0	0
F J	6 7	15	0	0				
J I	8.1	50	0	0				
V. H	7 4	8	0	0				
V. L.	6.5	34	6	0				
H J	9.5	60	0	0				
V. B.	5 5	32	0	0	13			
B. H.	9.5	45	0	0	10			
A. A.	9.5	35	0	0				
K. IJ	11.8	100	19	0				
M G	4.9	12	20	0				
R P	6.1	23	3	0				
O E.	9.5	31	2	0				

Number of days elapsed between withdrawal of iron and the new sternal marrow examination.

The period of time during which iron injections were administered.

Days elapsed between the last iron injection and the control sternal puncture

Methods

The technics described previously were used for staining and examining hemosiderin and sideroblasts (5) Serum iron was estimated by Brochner Mortensen's (8) or Laurell's (9) method For assessment of the total iron binding capacity of serum, Laurell's (9) or a slight modification of Ramsay (10) procedure was used Hemoglobin was determined as alkaline oxyhemoglobin.

Results

1 IRON DEFICIENCY ANEMIA

a) *Oral iron therapy* 11 patients with clinically verified chronic iron-deficiency anemia, whose bone marrow smears before treatment lacked hemosiderin as well as sideroblasts were re-examined after receiving a daily oral dose of 200 to 450 mg of iron for 2 to 22 days. Whereas

during and after interruption of oral iron therapy and after parenteral iron administration

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B. H.	9 5	45	0	0	10			
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				8	2,200 L m.	1	9 3	74	+
				52	1,500	1	12.1	90	+++
				4	1,000 L m.	17	8.9	15	+
				4	750 m.	21	8.3	23	+—
				4	400	1	7 8	55	+
				6	1,500 m.	9	11 5	60	+
				4	400	1	9.5	60	+
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before the commencement of iron therapy and a week after its cessation The sideroblast count was low on both occasions

c) *Parenteral iron therapy* Hemosiderin and sideroblasts were examined in marrow smears from 15 patients with chronic iron deficiency anemia, before and after parenteral iron therapy 10 of the patients received dextran iron (Imferon) intramuscularly in doses of 250 mg Fe, and 5 received intravenous injections of saccharated iron oxide (Intrafer) in doses of 100 mg Fe The total amount administered ranged from 400 to 2,500 mg Fe The injections were given over periods from 4 to 57 days, in most cases from 4 to 6 days. In 9 cases the control bone marrow aspiration was done the day after and in 6 cases from 7 to 130 days after the last iron injection Unlike the bone marrow smears from the group receiving iron by oral route, those from the patients treated with parenteral iron all but one contained iron staining granules in the reticulum cells (table I) Without exception the sideroblast count increased significantly In 2 cases, however the sideroblasts had risen merely to 15 % One of these patients suffered from rheumatoid arthritis and the other had actual small bleedings both conditions contributing to lowered sideroblast counts Patients M G who lacked reticular iron after therapy had received during 4 consecutive days a total dose of 1 000 mg iron-dextran i. m. and the marrow puncture was done on the fifth day The patient was bedridden, and delayed absorption from the site of injection cannot be ruled out. The marrow aspirate was poor in cells, a factor which also may have contributed to the lack of hemosiderin

2 ACUTE HEMORRHAGIC ANEMIAS

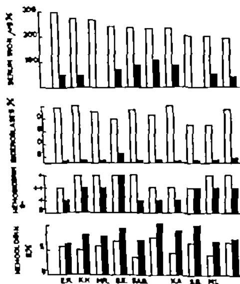
Fourteen patients with acute gastrointestinal hemorrhage had a normal hemosiderin content but had no or notably few sideroblasts in the bone marrow smears (table II) The low sideroblast count of these patients was often associated with a low serum iron concentration. None of the subjects had any signs of infection, and their E. S R. was normal In two of the above patients who were followed after they had stopped bleeding a rise in the hemoglobin concentration without exogenous iron supply was observed, indicating the presence of storage iron

3 PERNICIOUS ANEMIA AFTER COMMENCEMENT OF SPECIFIC THERAPY

Ten selected cases with megaloblastic anemia were examined with a control bone marrow aspiration during the initial phase of specific therapy with vitamin B_{12} or folic acid The sideroblast counts had dropped to subnormal levels in spite of the normal or enhanced hemosiderin content (fig 1) Concomitantly the serum iron concentration fell significantly from the high levels prior to treatment.

4 INFECTIOUS-TOXIC NON HEMORRHAGIC ANEMIAS WITH HYPOFERREMIA

The diagram in fig 2 illustrates the correlation between the sideroblast percentage and the hemosiderin content in 86 patients with infectious-toxic states and hypoferremia. The series includes 15 non-anemic cases Patients who had received iron or had had hemorrhage during the week preceding the sternal marrow aspiration were excluded The serum iron concentration did not exceed 50 µg % in any subject All had an elevated E. S R. The mean hemoglobin


Fig. 1 Megaloblastic anemia before (white columns) and after (black columns) commencement of specific therapy

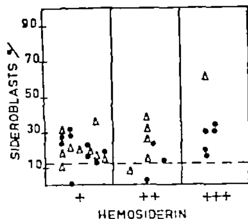


Fig. 2. The correlation between hemosiderin and sideroblast count in infectious-toxic anemia with hypoferremia. The serum iron concentration did not exceed 50 µg % in any subject. Δ designates cases with an infectious-toxic disease and hypoferremia but with hemoglobin value within the limits of normal.

Table II Illustrates the discrepancies between hemosiderin estimations and sideroblast counts which occur in cases of hemorrhagic anemia with actual bleeding The sideroblast counts are often associated with low serum iron concentration

Subject	Hb g %	Serum iron µg %	Sideroblast %	Hemosiderin 0—+++	Benzidine
R. A.	7.8	35	0	+	++
O. T.	8.1	53	8	+	+++
V F	6.7	55	4	+	++
V J	7.4	73	0	+	+++
K. O.	5.9	30	2	+	+++
K. P	10.1	59	10	+	++
K. P	10.5	20	8	+++	++
N. E.	10.2	33	6	++	++
H. H.	8.9	66	15	+	++
A. H.	6.1	30	0	++	++++
A. J	9.4	20	4	++	++
A. R. J	12.5	40	2	+	++
E. G.	10.2	—	2	+	+
J. A. E.	11.7	100	8	++	+++

concentration for the 71 anemic subjects was 9 g %.

All 66 patients had a normal or enhanced hemosiderin content. The sideroblast count was normal in 67 patients. However in 8 patients or in nearly 10 % of the entire group, less than 10 % sideroblasts were found, a

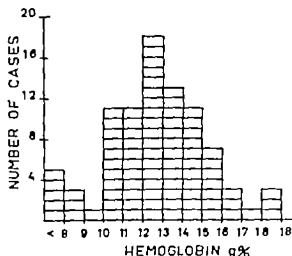


Fig 3 87 unselected cases who had a normal sideroblast count but lacked hemosiderin in the marrow smears, and who did not get iron therapy As is seen from the figure most of the patients had a normal hemoglobin conc. The anemic patients did not respond to iron therapy For details see text.

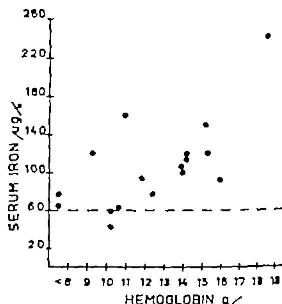


Fig 4 The figure presents the same group of patients as seen in fig 3 in whom serum iron determinations were done Low serum iron concentrations are not significantly correlated to a low hemoglobin concentration.

value usual in iron-deficiency states. In 11 patients the sideroblast count varied between 12 and 20 % i e a marginal value. The average sideroblast count for the whole group was 32 %

5 OTHER CONDITIONS WITH PRESENCE OF SIDEROBLASTS AND ABSENCE OF HEMOSIDERIN IN BONE MARROW SMEARS

Stainable iron was examined in all bone marrow smears submitted to the laboratory from the surgical and medical clinics during the period 1958—1960 After exclusion of subjects on iron therapy absence of hemosiderin with a concomitant normal sideroblast count was found in 87 subjects, i. e. in about 3 per cent of all marrow smears examined The material comprises 42 women and 45 men and is presented in fig 3 About one third of these marrow smears were poor in cells. It is seen that 67 patients or 80 percent of the group had a normal hemoglobin concentration whilst 20 patients had anemia but only 9 had a hemoglobin concentration below 10 g % Of these 9 patients 2 had accelerated red-cell destruction 2 myeloproliferative disease and 2 pernicious anemia 3 gave poor marrow specimens. It was sometimes difficult to detect hemosiderin in marrow smears from patients with myeloproliferative disease especially with total metaplasia The serum iron concentration was above 60 µg in 48 of the 53 cases in which it had been determined (fig 4) The patients with anemia in this group did not respond to iron therapy hence iron deficiency was not the limiting factor for hemoglobin production at the time of examination. Probably the severe anemia of some patients was due to a combination of previous iron losses and another supervening cause which at the time of examination was the dominating factor

Discussion

The results proved that patients with established iron-deficiency anemia regain a normal sideroblast count after as little as 2 to 6 days of oral iron therapy and that the sideroblasts disappear again if the oral iron supply is withdrawn for about a week provided the subject remains anemic. The studies show that a slight iron excess is enough to normalize the sideroblast count. A normal hemosiderin content with a concomitant low sideroblast count was found in acute hemorrhagic anemias and during the initial phase of specific treatment of megaloblastic anemias. The low sideroblast count in these conditions may be explained by an acute reduction of the readily utilizable iron pool due to either iron losses or to accelerated hemoglobin production. Although the stores are well filled iron cannot be mobilized from them at a rate high enough to meet the requirements of accelerated hemoglobin synthesis and at the same time supply iron excess for sideroblast formation. However not all bleeding patients, nor all patients with megaloblastic anemias after commencement of therapy showed the above-mentioned discrepancy between hemosiderin content and sideroblast counts. The sideroblast count is probably dependent upon the severity of hemorrhage, the degree of erythropoietic activity and the rate at which storage iron can be mobilized. If a state of equilibrium has been attained, as after cessation of bleeding or during the later stage of properly treated megaloblastic anemia, the mobilization of iron from the stores can keep up with the demands and the sideroblasts will rise spontaneously to normal levels. This is in accordance with the observation made by Kaplan et al. (11) in a case of megaloblastic anemia. The fall and rise of the sideroblasts was accompanied by a corresponding fall and rise in serum iron concentration. On the other hand, if slightly enhanced hemolysis with iron liberation takes place the sideroblasts might be found normal even if the subject is entirely deficient in iron. Apparently the "sideroblast-forming iron pool" is small and may undergo rapid changes with fluctuations in the total amount of hemoglobin, iron losses or exogenous or endogenous iron supply.

The sideroblast count alone, when examined during the unsteady state of iron metabolism has a limited value as a means of evaluation of iron stores. Absence of sideroblasts does not exclude well filled iron stores, and their presence merely indicates that for the moment some iron is available for erythropoiesis.

During the steady state of iron metabolism the absence of sideroblasts indicates that even the last reserves of storage iron have been exhausted. Because of the even distribution of the sideroblasts throughout the bone marrow their absence confirms a negative hemosiderin finding. Absence of hemosiderin with a normal sideroblast count should only be found before an anemia due to iron deficiency has developed. This constellation is found in normal non-anemic subjects whose iron stores are running out but are not completely exhausted (fig. 5).

Hemosiderin was absent after oral iron therapy in iron deficiency anemia even if as in one case iron was administered during 70 days. On the other hand, after parenteral iron therapy iron-staining reticular granules as well as a normal sideroblast count were generally found.

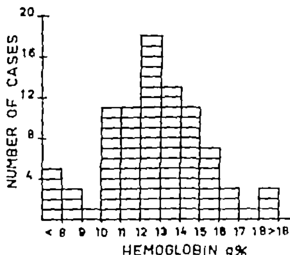


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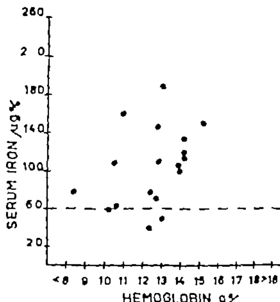


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depleted iron stores, because of the even distribution of sideroblasts.

In infectious-toxic anemia with hypoferremia the sideroblast count is usually normal, but in 10 percent of these cases it was found to be in the iron-deficiency range in spite of a normal or enhanced hemosiderin content.

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Coleman et al. (12) observed stainable reticular iron as little as 8 hours after an intravenous injection of 500 mg saccharated iron oxide. It must be questioned whether such iron staining particles encountered shortly after injections of colloidal iron are really hemosiderin or merely a phagocytosed colloidal iron complex. In our experience the iron particles seen after colloidal iron injections are of a peculiar type, uniform in appearance and consisting of small granules. Richter (13) has shown by electronmicroscopical studies on mice, that during the first 6 days after parenteral iron injections, iron is deposited in the tissues mainly as colloidal iron. About 3—4 weeks later endogenous ferritin and hemosiderin predominate and only traces of colloidal iron residues are present. Consequently we cannot infer from our experiments that naturally occurring storage iron in an amount equivalent to the smallest quantity administered, i. e. 400 mg iron as saccharated iron oxide, will be visible as hemosiderin in the bone marrow aspirate. Since native storage iron consists, to a large extent, of ferritin invisible by light microscopy, it is possible that storage iron has to be present in much larger quantities to be detectable in the form of hemosiderin in the marrow smear. The smallest dose of parenteral iron given, when as much as 3 weeks elapsed between the last injection and the sternal puncture, was 750 mg iron as iron dextran. After such an interval probably most of the colloid has been transformed into natural ferritin and hemosiderin.

Morse & Read (14) reported that sideroblasts may be absent in patients with hypoferremia and chronic infection in spite of a normal hemosiderin content. In contrast to what was previously reported (5), this disparity was now found in 10 per cent of patients of this category. The cause of it is obscure. It cannot be ascribed to accelerated hemoglobin production or to iron losses. Nor can the low serum iron level be a main cause, considering that the majority of cases in the same group with extremely low serum iron concentration had a normal sideroblast count. In infectious states the nonhemin iron may consist to a large extent of hemosiderin (15), which is less easily available than ferritin, causing a relative lack of the readily utilizable "sideroblast iron pool". For the differentiation of infectious anemias from iron-deficiency anemia, hemosiderin estimation is of greater diagnostic value than sideroblast examination, which may be misleading in about 10 % of cases.

Summary

The interrelationships between hemosiderin and sideroblast in bone marrow smears were studied experimentally and in a variety of clinical conditions. It has been shown that the "sideroblast forming iron pool" is small and readily utilizable and undergoes rapid changes. As a consequence the sideroblasts may be absent during acute hemorrhage and during the initial phase of specific treatment of megaloblastic anemia in spite of a normal hemosiderin content. On the other hand the sideroblast count is normal after a few days of oral iron treatment of iron-deficiency anemia.

In subjects with a normal hemoglobin concentration but with absence of hemosiderin, normal sideroblast counts may be found, revealing that iron is still available. If both hemosiderin and sideroblasts are absent, the lack of the latter is a valuable confirmation of quite

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Blood Plasma Level of Different Theophylline Derivatives Following Parenteral, Oral and Rectal Administration

By

B. Isaksson and B. Lindholm

Theophylline, a natural purine derivative, has been used for many years in clinical practice. It possesses, however, certain wellknown unpleasant characteristics. Given orally for example it tends to irritate the mucous membrane of the stomach and depending on its solubility in water (0.8 g per 100 ml at 25 C) it cannot be given parenterally (1).

For this reason a number of water soluble salts of theophylline have been introduced and used clinically the most common being theophyllamine (theophylline ethylendiamine, aminophylline *) (2). The weak acid theophylline is here combined with the organic base ethylendiamine and gives a readily soluble salt. The solution, however is very alkaline (pH 10) thus giving rise to irritation of tissues, particularly noticed after intramuscular administration. In acid gastric juice the weak acid theophylline will be displaced. Thus, theophylline ethylendiamine given orally has the same irritating properties as theophylline itself (3). More stable compounds are those where the base consists of choline or aminopropanol but the solutions are still alkaline.

An imposing number of attempts to reduce the disadvantages of the theophylline molecule have been performed by substituting different radicals for 7 H (4,5,6). These compounds give neutral solutions in water and are soluble in all proportions. They are also stable in both acid and alkaline media.

The aim of the present investigation was to compare two of these new derivatives 7 (2,3 dihydroxypropyl) theophylline, (DHPT) and 7(2-hydroxypropyl)-theophylline (HPT) with the classical theophyllamine (T) with reference to the blood plasma level after parenteral oral and rectal administration. The clinical and pharmacological effect will be discussed elsewhere (Lindholm, to be published).

Methods

Clinical methods

The amount of substance administered by the parenteral routes corresponded to 200 mg theophylline (i. e. 250 mg T 282 mg DHPT and 265 mg HPT) by the oral and rectal route to 500 mg theophylline respectively. Before the

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Methods

Clinical methods

The amount of substance administered by the parenteral routes corresponded to 200 mg theophylline (i. e. 250 mg T 282 mg DHPT and 265 mg HPT) by the oral and rectal route to 300 mg theophylline respectively Before the

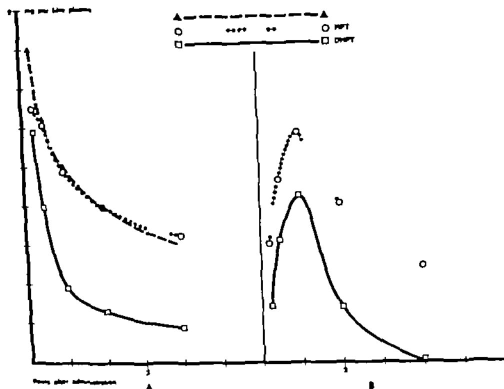


Fig 1 Mean plasma concentration of theophylline after parenteral administration of three different drugs in amounts corresponding to 200 mg theophylline A. Intravenous. B. Intramuscular

NaOH After centrifugation, measure the optical density at 273 mμ in 1 cm quartz cuvette against blank. In this study Zeiss spectrophotometer PMQII was used

For the quantitative isolation of *HPT* and *DHPT* respectively it was necessary to introduce further modifications. The main reason for this was their similar solubility in organic and inorganic solvents.

Add 1 ml 0.5 N NaOH to 5 ml plasma and shake for 5 minutes with 75 ml of the chloroform — isopropanol mixture (20 1) in separating funnel. Repeat the extration procedure once more then free the combined organic phase from water by shaking with 5 g sodium sulphate sicca. Filter and evaporate to 2—3 ml in vacuo. Transfer this small extract quantitatively to 25 ml test tube with ground joint and evaporate to dryness in vacuo. Dissolve the residue in 0.5 ml 1,2-dichloroethane, add 5 ml 0.1 N NaOH, insert the ground glass stopper and shake vigorously for 3—5 minutes. Centrifuge and measure the optical density of the alkaline phase at 273 mμ as above.

The initial alkalinization diminishes the co-extraction of interfering substances but has no influence on the extractability of the neutral compounds HPT or DHPT

A high ratio of organic phase to inorganic phase is necessary for quantitative extraction of the drugs from plasma. For the second transfer the reverse is true, hence the evaporation procedure is necessary The distribution coefficient is more favourable when substituting 1,2-dichloroethane for chloroform-isopropanol. Usually more than 97 per cent of the drugs were transferred in this step. The over-all yield was better than 93 per cent. The standard curve obeyed Beers law up to and far above the plasma level after toxic doses. The absorption peak was found at 273 mμ for HPT and 273.5 mμ for DHPT The optical density

Table I Plasma concentration of theophylline after intravenous intramuscular peroral and rectal administration of T DHPT and HPT together with blank values in a control group

Route	Drug	No. of pat.	Mean body weight and range kg	Mean plasma concentration and range in mg per litre after the different administration times (in min)					
				15	30	60	120	240	360
Intra venous	T	5	61.2 52 77	8.0 5 1—13 0	6.2 4.0—9.2	3.0 2.7—9.2	4.0 0.5—6.7	3.0 0—3 6	
	DHPT	6	61.9 52–90	5.9 4 4—9.2	4 0 2.1—6.4	1.9 0.8—3.6	1.3 1.2—1 7	0.9 0.1—1.6	
	HPT	7	59 1 45–76	6.5 4.5—8.0	6.1 3 1—8.0	4.9 1.9—6 0	4 0 2.5—6.3	3.2 0.6—5.6	
Intra muscular	DHPT	4	62.1 50–75	1 4 1 4—2.4	3 1 2.2—3.6	4.2 3.0—5.0	1 4 1.2—2.0	0 0—0	
	HPT	6	60.7 54–75	3.0 1.0—4 0	4.6 2.2—6.4	5.8 4.2—7.2	4.0 2.6—5.0	2.4 1.2—3.4	
Oral	T	9	69 7 51 98	3.4 0.8—8.6	7.8 2.8—10.6	7.2 3.0—9 4	7.0 4.8—8.4	4.9 3.3—6.8	2.8 0.8-3.6
	DHPT	10	66.2 43–86	2.4 0—7 0	5.2 4 4—8.2	4 1 1.8—6.0	3 4 1.2—6.4	1 4 0.8—2.2	0.2 0-1.0
	HPT	10	70.6 60–83	4 1 1.2—7.8	7.8 4 1—11 0	7.6 5.5—10.4	6.8 4.2—8.4	4.9 3.8—6.0	1.8 0.8-2.6
Rectal	T	4	66.0 59–80	0 0—0	1.6 0 7—3.2	3.2 2.2—4 0	3.1 4.3—5.6	6.4 5 4—7 4	4.9 4.0-6.0
	DHPT	4	66.7 57 75	0 0—0	0 0—0	0.3 0—1 0	1.5 1 0—2.4	2.9 1.8—4.2	3.9 2.3-3.6
	HPT	4	61 4 56–63	0 0—0	1 7 1.6— .6	4.3 3.0—5 4	5 7 3.8—7.0	7 7 5 0—11 6	7 4 7.0-10.2
Control		4	48.1 47–69	+0.8 −0.6 −2.0 +1 9 ±0.0	+1.0 +0 8 −0.3 +0.2 +0.4	+1.8 +0.3 −1 0 +1 4 +0 6	+0.3 −1 0 +0 1 +0 6 ±0 0	+1 4 — +0.9 +0 1 +0.8	

parental administration the drug was dissolved in 10 ml of saline. The injection by vein was performed over a period of 4 minutes.

The tablets were uncoated and contained all the same constituent. The same suppository base (Witepsol H) was used with all derivatives. Special caution was taken in order to make the rectal administration as uniform as possible. Table I gives the number of patients in each group, the body weight data and times for the blood sampling A blank sample was always taken before the administration.

Chemical methods

The blood samples, collected in heparinized test tubes were centrifuged. Only haemolysis free plasma was used for the analyses.

For the determination of *theophyllamine* a slight modification of the method of Shack and Waxler (7) was used.

Shake plasma twice with a 20 1 mixture of chloroform and isopropanol. Remove the water droplets from the organic phase by shaking with sodium sulphate sicca. Extract the drug from the organic phase by shaking with 0 1 N

according to Hald (8) The maximum point is given by differentiating the regression equation with respect to t which leads to

$$t_{max} = -a \qquad (3)$$

From the data obtained after intravenous administration it seemed possible to describe the curve as the exponential function

$$y = k\,t^{a} \quad t > 0 \qquad (4)$$

which also is linear expressed logarithmically and the parameters may thus be determined as above.

Results

The results are given in table I and figs. 1—2 which show the observed means at different times after administration.

In table II are given the parameters found and inserted into the mathematical regression equation used for the statistical treatment.

The optical density of the plasma blanks taken before administration varied considerably between individuals. The observed variations on one individual during a period as long as the experimental period was, however limited, as judged from the 4 cases in group 5 see table I

The plasma curve for HPT follows close to that for T throughout the experiment after *intravenous administration*. The curve for DHPT differs significantly (t = 5.50) from the two others, and show slow plasma concentration already 30 minutes after injection.

The curves after *peroral administration* of a dose 1.5 times the parenteral one shows maximal values after ½—1 hr The maximal concentration corresponds to that observed about 15 minutes after intravenous injection. There is no significant difference between HPT and T but the curve for DHPT differs significantly (t = 4 70) from both.

After *rectal administration* also both HPT and T show rather high maxima, but much later than after peroral loading. The

Table II The parameters k, and b in equations (1) and (4) as determined from the experimental results

Route	Drug	k	a	b
Intravenous	T	5 0	−0.37	—
	DHPT	2.2	−0.69	—
	HPT	4 8	−0.18	—
Intramuscular	DHPT	6.2	−1 48	1.83
	HPT	10 8	−0.67	0.79
Peroral	T	13 4	−0 67	0.78
	DHPT	5 2	−0.54	0.11
	HPT	14.0	−0 54	0.70
Rectal	T	6.1	−0.57	1 75
	DHPT	0.78	1.20	4.55
	HPT	3 4	0.[illegible]5	1.87

DHPT concentration still rises after 6 hours, but at a significantly lower level (t = 4.80) No significant difference between HPT and T appears.

Intramuscular administration finally gives maxima after 1 hr for both HPT and DHPT DHPT reaches here the same range as after peroral administration of 50 % higher dosage, but the curve level is significantly lower than that of HPT (t = 4 38)

Discussion

The similarity between the curves for T (theophylline ethylendiamine) and HPT (β-hydroxypropyl theophylline) makes it possible to assume that these drugs are handled in the same manner in the body regardless of administration route. The observed lower plasma levels DHPT (dihydroxypropyl theophylline) suggests a decreased inflow to and/or increased outflow from the plasma compartment as compared with the two other derivatives. The initial phase of the curves obtained after peroral administration seems to indicate a rather similar inflow velocity for the three drugs. Even after

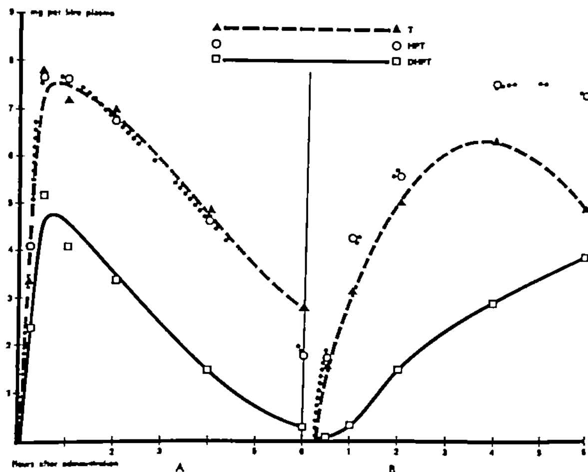


Fig 2. Mean plasma concentration of the theophylline after administration of three different drugs in amounts corresponding to 300 mg theophylline. A. Peroral. B. Rectal.

due to the drug administered was obtained by subtracting with the plasma blank value. All concentrations in the present study are expressed as mg theophylline per litre of plasma.

Statistical methods

From the individual plasma curves in this study it seemed possible to conclude that T and HPT behaved similarly while DHPT every time showed apparently lower plasma concentrations. For statistical treatment it was, however difficult to use the individual curves. It seemed therefore logical to construct mathematical models, which fell close to the mean curves and had parameters, determined from the findings.

The regression model for the experiments with peroral, rectal and intramuscular administration should show an initial increase in plasma concentration, being zero at time zero, and then increasing gradually until a maximum is reached, i. e. when inflow and outflow from plasma coincides. Thereafter a gradual fall in the curve back to zero should appear

A regression equation, which fitted well in with the results obtained, was

$$y = k\,e^{at}\,t^{b} \quad t > 0 \tag{1}$$

where
t = time in hours after the administration
y = the calculated concentration at time t
k, a and b = parameters determined from the experiments.
Equation (1) may be transformed into

$$\ln y = \ln k + at + b \ln t \tag{2}$$

where t and ln t are th independent variables.

It is thus possible to estimate the parameters and analyze the results on a linear function

according to Hald (8) The maximum point is given by differentiating the regression equation with respect to t which leads to

$$t_{max} = -\frac{b}{a} \qquad (3)$$

From the data obtained after intravenous administration it seemed possibl to describe the curve as the exponential function

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which also is linear expressed logarithmically and the parameters may thus be determined as above.

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rectal administration the resorption of the drugs seems to be similar The apparent deviation of the DHPT curve from the others after intravenous administration clearly demonstrates the higher outflow of this drug from the plasma compartment.

The plasma level after administration of T has been studied previously by among others, Waxler & Schack (9) and Brodwall (10) Our curves reveal a more pronounced decline than theirs, but it should be borne in mind that these authors examined whole blood and in general used different quantities of drugs. The theophylline molecule is said to be restricted to plasma (Schack & Waxler 7) hence it seems more adequate to analyse plasma instead of whole blood. Moreover our curve after oral administration has the same appearance as that of Turner Warwick (11) who also examined plasma

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Plasma levels for HPT have not been reported previously The high plasma concentration obtained regardless of administration route, combined with the chemical properties, mentioned in the introduction, justifies further clinical studies.

Summary

The plasma levels of three theophylline derivates after parenteral, oral and rectal administration have been studied.

The drugs compared were the classical alkaline and unstable theophylline ethylendiamine and the new neutral and stable compounds, dihydroxypropyl theophylline and β-hydroxypropyl theophylline.

Almost identical curves for theophylline ethylendiamine and β-hydroxypropyl theophylline were found as to height and duration, whereas dihydroxypropyl theophylline consequently showed statistically lower levels.

A method for the determination in plasma of the two neutral compounds was described.

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Acute Renal Failure Following Intravenous Pyelography with Hypaque

By

NEVILLE BERLYNE and G. M. BERLYNE

Intravenous pyelography is a valuable investigation in diseases of the renal tract. It is moderately safe and only rarely causes fatal reactions although non-fatal reactions are known to occur few have been documented. The purpose of this paper is to draw attention t the occurrence of an episode of acute renal failure due to intravenous pyelography (I V P) with "hypaque" (sodium dia trizoate)

Case history

J L., 67-year-old retired labourer was admitted to St. Thomas Hospital, Stockport, on 2.2.60, suffering from auditory hallucinations without clouding of consciousness diagnosis of late paraphrenia was made. I addition he had precipitancy of micturition and nocturia for five years he had suffered from hesitancy for tw years. He had no past history of allergic diseases. His bladder was distended to the umbilicus. On rectal examination his prostate was small and firm. His blood pressure was 150/70. Blood urea was 67 mg/100 ml. Urinalysis demonstrated no albuminuria or glycosuria. S. G. of urine 1,020. H was able to pass his urine freely his daily urine volume varying from 600—1,800 ml. After surgical advice an intravenous pyelogram was carried out on 16.2.60, 20 ml of 45 % "hypaque" (sodium-diatrizoate) being injected slowly intravenously without any prior sensitivity test half an hour after the injection he began to shiver and felt ill. H had nausea and abdominal pain. His pulse rate was 124 per minute and his blood pressure, at first 120/80, fell to 60 mm systolic by next morning. H passed only 168 ml of urine in the next twenty-four hours. His blood urea had risen to 100 mg/100 ml t this stage. A catheter was passed and 1,290 ml of residual urine was drained off A noradrenaline intravenous drip (16 mg to the pint of 5 dextrose) was set up but failed to raise his systolic blood *pressure above 60 mm. Hydrocortisone 100 mg* was given intravenously and "aramine" (metaraminol) was given intravenously and the concentration increased until it was 30 mg per pint of 5 % dextrose, the blood pressure being maintained at 100 mm systolic. In the next twenty-four hours he passed 300 ml of urine and the drip was discontinued. An E. C. G taken at this time was normal. Oral prednisolone 10 mg six hourly was substituted for the hydrocortisone. In addition the patient was placed on modified Bull

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of fatal anuria in these patients following these procedures (Bartels et al 1954 Myhre et al. 1956) It is postulated that anuria in myelomatosis following I. V P is due to a combination of the effects of the induced dehydration and the abdominal compression.

The patient of Bartels et al. (1954) received 20 ml of 50 % diodrast for intravenous pyelography In the 11 cases reported by Alwall et al. 9 were caused by i.v diodrast in concentrations of 35 % to 50 % while the remaining two cases received methiodal sodium. Both patients of Myhre et al. (1956) received 20 ml of 30 % diodrast Prendergrass et al. reported 25 immediate fatalities of which 21 were due to diodrast sodium iodomethamate was responsible for 3 of the deaths, and urokon sodium for one fatality Landmann et al. in 1959 reported a reaction to 35 % neo-iopax.

We have been unable to find another report of acute renal failure following the use of hypaque for intravenous urography nevertheless the manufacturers of hypaque suggest that an intravenous test dose be given before the main injection in order to prevent serious reactions occurring. Although Prendergrass et al. (1955) found this to be useless in 15 out of 25 patients there is still an appreciable proportion in whom it is well worth carrying out a sensitivity test. There remains the possibility that sensitivity would have been detected in our patient had the test been used and he would have been spared an episode of acute tubular necrosis.

Summary

A case of non-fatal acute renal failure following intravenous urography with hypaque is described Complications of I. V P are rare but may be fatal. No patient with myelomatosis should be *subjected to this investigation* in view of the increased risk of tubular necrosis a history of allergy is also a contraindication to intravenous pyelography All patients should be tested for sensitivity to the contrast medium before urography

Acknowledgements

Thanks are due to Prof. D. A. K. Black for permission to publish this case and for *criticising* the manuscript. We should also like to thank Dr Russell Young under whose care the patient was originally admitted to hospital.

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regimen 10 % glucose orally in daily volume equal to 600 ml plus urine volume By 19.2 60 his blood urea was 245 mg/100 ml. Urine output had risen to 760 ml in twenty-four hours. The urinary loss of electrolytes (75 mEq of sodium 60 mEq of potassium per day) was replaced orally with third normal saline and mist. pot. cit. in the appropriate amounts, varying according to the preceding day s loss. Eight days after the I V P his urine volume was 1 400 ml and the next day it reached 2 160 ml, the blood urea falling to 207 mg/100 ml he was transferred to Manchester Royal Infirmary under the care of Prof D A K Black, on 25.2 60 Serum sodium at this time was 137 mEq/litre, potassium 4 7 mEq/l and plasma bicarbonate 31 mEq/l. The intravenous pyelogram was reported as showing early calyceal hydronephrosis bilaterally with a bladder fundus enlarged to L.3 By 29 2 60 the blood urea had fallen to 83mg/100 ml and on 19.3 60 it was 64 mg/100 ml. Prednisolone was reduced gradually His urine output fell gradually to between 1,200 and 1 700 ml per day A transurethral prostatectomy was carried out by Mr T Moore and he gradually made a complete recovery

Discussion

Any investigation which carries even a remote possibility of death or morbidity should only be undertaken after careful consideration of the benefits likely to accrue to the patient from the investigation and whether these outweigh the risks. Intravenous pyelography is no exception to this rule, although fatal reactions following this procedure are rare thus Prendergrass et al (1955) reported 31 fatal reactions out of a total of 3 800 000 urograms during the period 1942—1952 the incidence of non fatal reactions has never been determined. In 15 out of 25 cases Prendergrass et al found that the use of sensitivity tests using the urographic medium had given false negative results they thought that a positive history of allergy was more reliable than prior sensitivity testing Nevertheless in our patient no sensitivity test was done and there was no allergic history had a test of sensitivity to hypaque been performed it is possible that the patient would have been able to avoid an episode of acute renal failure as urography would not have been proceeded with.

Prendergrass et al. (1955) found that an elevated blood urea predisposed to reactions to intravenous urography Unfortunately the I V P is an important investigation in kidney disease in which the blood urea is moderately or slightly raised however there is little point in intravenous urography if the blood urea is over 100 mg/100 ml for with renal failure of this degree it is highly improbable that there will be any contrast between the renal pelvis and surrounding tissue presumably the prolonged high blood levels of contrast medium following I V P in these patients increase the risk of a reaction to the urographic drug In our patient the blood urea was 64 mg/100 ml indicating moderate impairment of renal function The reaction was delayed half an hour following which he developed the characteristic malaise, hypotension and abdominal pain (Alwall et al 1955 Landmann et al 1958) The resulting acute renal failure was found to be due to acute tubular necrosis in all seven fatal cases described by Alwall et al. (1955) this may follow the hypotension and be due to renal ischaemia but it may occur in patients who do not experience an episode of hypotension as in the case of Landmann et al. (1958)

Multiple myeloma is an absolute contraindication to intravenous urography and also to retrograde pyelography in view of the increased chance

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rom Frederiksberg Hospital, Medical Department E (Head N I. Nissen, M. D) and the Central Laboratory (Head: A. Lenn-Nielsen, M. D), Copenhagen, Denmark

Effect of Triiodothyropropionic Acid (Triopron) on Hypercholesterolaemia

By

Th. Friis, J Lintrup and N I Nissen

Since Gross and Pitt Rivers (11 12) identified l-triiodothyronine in human serum and found it to be 4—5 times as active as l-thyroxine in raising the basal metabolic rate in myxoedema, great efforts have gone into developing other thyronine derivatives and studying their effects.

Thus, the acetic acid analogues of thyronine and triiodothyronine have been intensively studied since 1953 especially after triiodothyroacetic acid had been identified in homogenates of renal tissue following injection of triiodothyronine into rats (1 2, 20) Some authors believed that now the true active principle of the thyroid hormone had been found, as triiodothyroacetic acid was believed to be able to increase the oxygen uptake of tissue slices *in vitro* (23) However this has not been confirmed by others (4 13 26)

In 1956 Lerman et al. (17) reported that in patients with myxoedema triiodothyroacetic acid could lower the serum cholesterol without materially raising the basal metabolic rate (B. M. R.) Since then, the effect of several thyroxine derivatives upon serum cholesterol has been investigated. In a certain dosage, some of them have proved capable of lowering the serum cholesterol without materially affecting the B. M. R. (7 13 14 18, 19) D-thyroxine is said to exert a similar effect (22)

Since the effect of triiodothyropropionic acid upon serum cholesterol in the human body has been studied by only a few authors and on relatively few patients over short periods (5 13 19 25) we undertook a study of this aspect in hypercholesterolaemic patients.

Material and method

The effect of triiodothyropropionic acid ("triopron") upon serum cholesterol was studied in 6 patients with myxoedema and 26 euthyroid patients with hypercholesterolaemia of different aetiology The dosage was

Triopron was kindly supplied by Messrs. H. Lundbeck & Co. Ltd Copenhagen.

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patients also had determination of labelled triiodothyronine uptake by the red cells (8) and 7 had a thyrotrophin stimulation test (10).

The patients received an ordinary diet, but it was enjoined upon them to avoid foods with high content of cholesterol or of animal fat.

Analytical method for serum cholesterol

All blood samples were collected before breakfast.

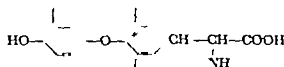
Total cholesterol in the serum was determined by a micromethod advocated by Kingsley & Schaffert (15) but slightly modified. This method is quick and well suited for serial analysis, as it does not include extraction and saponification procedures. With 200 µl serum added to 10 ml chloroform, the water content of the serum may be removed by adsorption onto dry magnesium sulphate. This is followed by Liebermann-Burchard reaction on the chloroform solution. Coloured substances from the serum are removed beforehand by adsorption onto Fuller's earth. The colour thus produced is stable from the 3rd to the 10th minute. Extinction at 625 nm was read in Beckman DU spectrophotometer at the end of 5 minutes. Each serum was analysed in duplicate. Each analytical series included a cholesterol solution of known concentration.

On the basis of 100 duplicate analyses of various sera, the standard deviation for a single

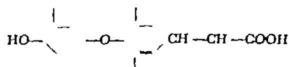
patients

BMR (− 10 to + 10%)				PBI (3.0—8.0 µg%)				T uptake (6.0—10.5%)	Pulse rate	Weight loss (kg)	Comments
	b		d		b		d				
− 33	+ 69	+ 33	9	0.8	+ 17.1	+ 9.4	14	4.3→ 9.0	66→99	− 4	Myxoedematous→euthyroid
− 33	+ 51	+ 41	7	0.8	+ 6.6	+ 3.3	5	9.0→10.5	90→66	+ 4	Euthyroid
− 33	+ 59	+ 35	11	5.1	+ 10.0	+ 5.0	10	5.0→ 9.4	63→85	− 8	Myxoedematous→euthyroid
− 16	+ 23	+ 21	5	3.6	+ 4.8	+ 4.6	4		100→69	− 3	Myxoedematous→euthyroid
	85				2.6			6.1	60		Euthyroid
− 15	+ 37	+ 13	10	2.6	+ 12.5	+ 6.6	19	6.1→ 6.3	50→78	− 2	*Euthyroid*
	86				5.6				72		Euthyroid
− 14	+ 19	+ 3	6	5.6	+ 11.0	+ 4.4	3		73→56	− 2	Euthyroid
	112				5.3				70		Euthyroid
+ 12	+ 10	+ 1	6	5.3	+ 2.7	+ 1.1	4		70→68	+ 1	Euthyroid

Alteration from pre-treatment value to mean value during treatment. d = Number of determinations



3,5,3'-triiodothyronine
(TERTRONIN)



3,5,3'-triiodothyropropionic acid
(TRIOPRON)

Fig. 1

2—6 mg daily. Fourteen patients had a history of coronary thrombosis within the past 6 months to 5 years. In order to obtain a comparison with the effect of current thyroid drugs, treatment with desiccated thyroid was given to 4 of the myxoedematous patients for a period, and treatment with l-triiodothyronine (tertroxin) to 2 of the triopron-treated euthyroid patients and another 6 patients with hypercholesterolaemia and coronary sclerosis.

In addition to ordinary clinical examination all the patients had the following tests once or twice a month: Determination of the B. M. R., of protein-bound iodine by the method of Barker (3) and of serum cholesterol by the method of Kingsley & Schaffert (15). Twelve

Table I. Effect of triiodothyropropionic acid (triopron) upon the serum-cholesterol in 6 myxoedematous

Case	Sex	Age	Dose (mg)	Duration of treatm.	Cholesterol (150—300 mg/100 ml)			
					a	b	c	d
1	F	71	3—5 (triopron)	5 months	473	— 378	— 270	15
			120 (desiccated thyroid)	6 months	473	— 317	— 266	7
2	F	60	4 (triopron)	6 months	577	— 335	— 276	11
3	M	56	2—4 (triopron)	2 months	396	— 217	— 142	11
4	F	67	60 (desiccated thyroid)	3 years	522			
			2—6 (triopron)	11 months	522	— 379	— 235	36
5	F	64	120 (desiccated thyroid)	16 years	336			
			2—4 (triopron)	7 months	336	— 144	— 14	6
6	F	66	120 (desiccated thyroid)	3 years	426			
			2 (triopron)	6 months	426	— 237	— 207	6

The letters under cholesterol, BMR and PBI indicate: a = N. treatment. b = Maximum alteration during treatment. Before treatment there were three determinations.

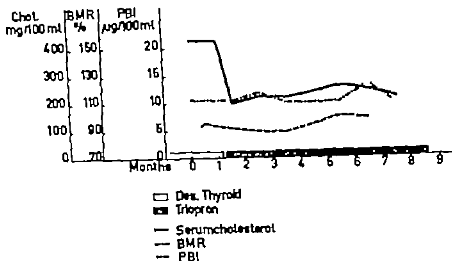


Fig 3. Case 6 in table I. Course of serum cholesterol, BMR and PBI

mine it was only 82 mg/100 ml (53 141 15 128 mg/100 ml)

In case 1 the treatment was altered to desiccated thyroid, 120 mg daily at the end of 5 months in order to compare the effect with that of triopron. While the mean B. M. R. was now + 8 % as compared with 0 on triopron the mean cholesterol was 207 as against 203 mg/100 ml. The results are illustrated in fig 2. Since, however the triopron therapy was interrupted for a month, it is difficult to compare the results, but the course of the curves indicates that triopron in the dosage used exerted a greater effect than desiccated thyroid upon the serum cholesterol.

In the myxoedematous patients who had previously been treated with desiccated thyroid (cases 4 5 and 6) the serum cholesterol fell after desiccated thyroid had been replaced by triopron. This was particularly marked in case 6 whose mean cholesterol value fell by 207 mg/100 ml without any increase in B. M. R. (fig 3) Case 4 also showed a considerable fall of cholesterol (235 mg/100 ml) but in this case the B. M. R. rose (+ 13 %) In case 5 the cholesterol fell but slightly (14 mg/100 ml)

All the patients treated with triopron lost weight with the exception of case 6 In case 4 who had previously been on desiccated thyroid the T uptake by the red cells did not alter while in cases 1 and 2 it returned to normal during triopron therapy In all but one (case 6) the PBI rose to above normal values on triopron medication. None of the patients showed thyrotoxic symptoms at any time. Triopron did not give rise to any side effects.

2 Euthyroid patients

As already mentioned, 26 euthyroid patients were treated with triopron. Fourteen had a history of coronary thrombosis 6 months to 5 years previously (cases 1—14) case 6 also had xanthelasmata. The others, except cases 15 23 24 and 26 had coronary sclerosis with a tendency to anginal attacks on exertion. Two of them (cases 19 and 21) were probably suffering

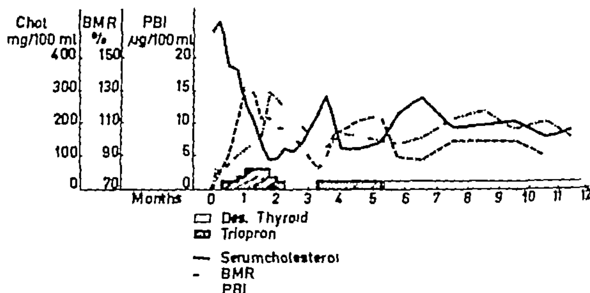


Fig. 2. Case 1 in table I. Course of serum cholesterol, BMR and PBI.

analysis was calculated as ± 8 mg/100 ml which is ± 6 mg per cent for a duplicate determination. In 30 duplicate analyses of the same known solution carried out by different technicians on 30 different days, the standard deviation was ± 7 mg/100 ml.

Results

The results are shown in tables I—VI and figs. 2—7.

1. Myxoedema

Table I gives the result of treating the 6 myxoedematous patients.

Cases 1, 2, 3 were newly diagnosed and had not previously been treated. All were of a typical myxoedematous appearance and had a low B. M. R. In cases 2 and 3 the PBI was normal, while in cases 1 and 2 the triiodothyronine uptake by the red cells was low.

Cases 4, 5 and 6 had been on thyroid medication for several years. Nevertheless, the serum cholesterol was elevated. In case 4 the diagnosis was confirmed by a thyrotrophin stimulation test (10). When the investigation was started all three were clinically euthyroid, but the B. M. R. was slightly below normal in cases 4 and 5.

In the 3 newly diagnosed cases the myxoedematous changes yielded completely to triopron. The pulse rate went up, the patients lost weight and felt better. All showed a marked decline of cholesterol (142—276 mg/100 ml) and the B. M. R. returned to normal. The dosage, 2—5 mg daily, was tolerated without any side effects. The effect was manifest within a week or so, unlike desiccated thyroid which requires several weeks to reach its maximum effect. When the decline of cholesterol in the fresh myxoedematous cases on triopron (table I, cases 1, 2, and 3) was compared with the decline of cholesterol in another four fresh cases of myxoedema treated with triiodothyronine and published elsewhere by one of the authors (9), that obtained with triopron was considerably more marked. In the 3 triopron-treated patients the mean decline was 229 mg/100 ml (270, 276, 142 mg/100 ml), while in the 4 patients on l-triiodothyro-

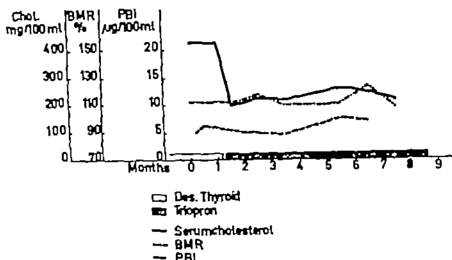


Fig. 3. Case 6 in table I Course of serum cholesterol, BMR and PBI.

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Table II Effect of triiodothyropropionic acid (triopron) upon the serum-cholesterol in euthyroid patients with

Case	Sex	Age	Dose (mg)	Duration of treatm.	Cholesterol (150–300 mg/100 ml)			
					a	b	c	d
1	F	55	120 (desiccated thyroid)	1 year	351			
			2—4	7 months	351	−162	−69	19
2	M	40	2	9 months	378	−222	−156	20
3	F	66		6 months	324	−198	−118	14
4	M	57	2—4	5 months	362	−220	−77	16
5	M	50	6	11 months	390	−171	−80	26
6	M	53	2	10 months	474	−247	−85	49
7	M	55	8	6 months	312	−52	+43	23
8	M	56	2	4 months	382	−151	−93	9
9	F	57	4—6	7 months	329	−293	−82	19
10	F	64	2	6 months	396	−192	−150	14
11	F	64	2—4	7 months	404	−186	−134	16
12	M	52	2	11 months	441	−260	−114	27
13	F	63	2—4	9 months	331	−270	−162	21
			40 µg (tertroxin)	3 months	470	−17	−13	3
14	M	49	4—6	13 months	467	−165	−56	24
15	F	61	120 (desiccated thyroid)	3 years	425			
			2—4	8 months	425	−171	−81	9
16	F	64	4	9 months	418	−103	−101	22
17	M	63	2—6	7 months	297	−118	−45	18
18	M	78	—4	8 months	372	−164	−102	12
19	F	55	2—4	6 months	670	−160	−73	29
			40 µg (tertroxin)	3 months	609	+104	+98	3
20	F	57	2—3	9 months	331	−139	−59	32
21	M	42	2—4	10 months	422	−179	−79	24
22	M	53	4	3 months	518	−183	−79	15
23	F	41	2—4	6 months	359	−112	−23	6
24	M	57	2—6	8 months	316	−146	−54	13
25	M	46	2—4	6 months	264	−112	−91	7
26	M	49	4—6	4 months	326	−137	−86	5

a = No treatment. b = Maximum alteration. c = Alteration from pretreatment value to mean value during

hyperchelesterolaemia

BMR (−10 to + 10%)				PBI (3.0—8.0 μg%)				T_3-uptake (6.0—10.5%)	Pulse rate	Weight loss (kg)	Comments
	b		d	a	b		d				
91				6.9					70		
− 9	+ 26	+ 8	8	6.9	+ 4.4	+ 1.2	9		70→76	0	
+ 6	+ 6	− 2	4	7.0	+ 3.8	+ 1.3	10		52→66	− 7	
+ 21	+ 9	− 2	6	5.9	+ 20.0	+ 13.6	3		76→96	− 4	
+ 8	− 15	− 9	4	5.8	+ 13.6	+ 5.0	6		85→65	+ 4	Paroxysmal tachycardia
+ 22	+ 22	+ 6	8	6.4	+ 8.5	+ 6.4	14		68→82	− 3	
+ 22	+ 13	− 6	5	6.0	+ 12.2	+ 6.6	4		76→80	− 3	Xanthelasmata diminished
+ 13	+ 23	+ 10	11	6.8	+ 4.9	+ 1.3	7	6.7→ 8.4	66→64	0	
− 5	+ 14	+ 6	7	5.2	+ 15.2	+ 4.9	6		80→60	− 1	
+ 9	+ 26	+ 4	6	6.3	+ 16.3	+ 8.0	11		72→72	− 2	
+ 4	+ 17	+ 7	4	5.3	+ 16.3	+ 3.9	11		65→74	− 6	
+ 24	+ 8	− 3	5	6.7	+ 11.3	+ 5.8	8		70→68	− 3	
+ 21	+ 46	+ 7	8	6.8	+ 8.2	+ 1.9	15		76→70	− 4	+ Diarrhoea + Anginal attacks
+ 10	+ 34	+ 28	2	3.4	+ 14.1	+ 3.3	11		75→84	− 7	
− 8	+ 4	+ 0	2						68→66		
+ 13	+ 15	+ 4	3	4.8	+ 22.0	+ 11.5	8		86→90	0	
113				6.9				7.5	73		
+ 13	+ 35	+ 10	7	6.9	+ 3.2	+ 2.2	6	7.5→ 6.8	73→71	− 2	
+ 22	+ 19	+ 4	7	4.8	+ 8.0	+ 5.3	12		66→66	− 4	Palpations
+ 28	+ 33	+ 14	8	6.2	+ 12.8	+ 6.6	10	7.6→10.7	62→70	− 4	Xanthelasmata unchanged
+ 24	+ 13	− 1	10	3.7	+ 19.9	+ 8.2	6	6.7→ 7.4	68→68	0	Goitre
+ 18	+ 23	− 3	9	6.2	+ 18.5	+ 4.2	13	8.8→ 8.8	65→65	0	Anginal attacks
− 7	+ 4	+ 0	3	5.1	+ 2.1	+ 2.1	3		56→56	0	Xanthelasmata Xanthomas
+ 13	+ 45	+ 9	10	6.7	+ 15.1	+ 3.0	12	6.9→ 9.6	68→70	− 3	Xanthelasmata
− 1	+ 21	+ 8	3	3.6	+ 17.3	+ 9.7	10		49→49	0	Xanthelasmata Xanthomas
− 5	+ 13	+ 4	5	4.3	+ 12.8	+ 4.1	7		60→65	− 2	Anginal attacks
+ 7	+ 37	+ 13	5	5.1	+ 3.7	+ 1.9	4	6.7→ 9.6	57→72	+ 5	Nervousness
− 9	+ 7	4	4	5.6	+ 20.3	+ 6.8	8		64→78	− 1	
+ 7	+ 42	+ 13	7	5.5	+ 4.6	+ 1.2	7		59→67	− 2	
− 8	+ 23	+ 17	4	6.8	+ 12.0	+ 6.8	4	10.6→9.7	65→72	− 6	

treatment. d = Number of determinations during treatment. Before treatment there wer three determinations.

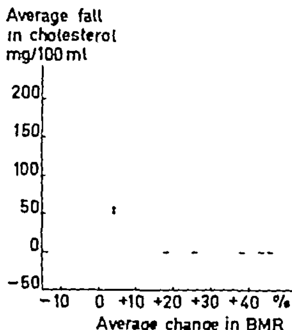


Fig 4 Relation between mean increase in B.M.R and mean decline of serum cholesterol in triopron-treated euthyroid patients.

from Müller Harbitz disease (xanthomas, hypercholesterolaemia coronary sclerosis, but uncertain heredity) and another two (cases 17 and 20) had xanthelasmata. Case 15 was neurotic, case 23 had chronic pyelonephritis, and cases 24 and 26 were obese. The serum cholesterol was elevated in all but cases 17 and 25 in whom it was at the upper limit of normal.

Table II gives the results. In 2 cases a *mean decrease in cholesterol of 150—200* mg/100 ml was obtained (cases 2 and 13) One of them showed a considerable increase in B M R. (case 13 + 28 %) In the other patient the B M R. dropped *very slightly (—2 %) In 6 the cholesterol* fell by an average of 100—150 mg/100 ml (cases 3 10 11 12 16 and 18) and among them the B M. R. did not rise by more than 10 % In three (cases 10 12, *and 16) there was a rise of 0—10 %* and in 3 a slight fall (cases 3 11 and 18)

In 13 patients (cases 4 5 6 8 9 14 19 20 21 22 24 25 and 26) the cholesterol fell by 50—100 mg/100 ml The *increase in the B M. R. exceeded 10 %* in two (cases 25 and 26) in 8 (cases 5 8, 9 14 20 21 22 and 24) it was between 0 and 10 % while in 3 there was a slight fall (cases 4 6 and 19) Lastly the cholesterol fell by from 0 to 50 mg/100 ml in two patients (cases 17 and 23) whose B M R rose by 10—20 % In one (case 7) the cholesterol rose during the treatment.

There was no definite relation between a possible increase in the B. M R. and a decrease in cholesterol or between the cholesterol level before the treatment and

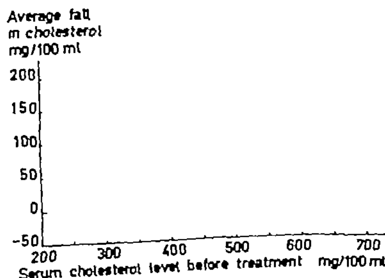


Fig 5 Relation between pretreatment serum cholesterol level and mean decline of serum cholesterol during treatment of euthyroid patients with triopron.

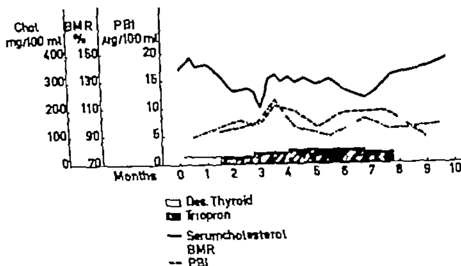


Fig. 6. Case 1 in table II. Course of serum cholesterol, BMR and PBI

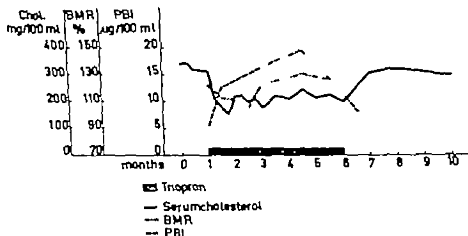


Fig. 7 Case 3 in table II Course of serum cholesterol, BMR and PBI

the decrease in cholesterol during the treatment (figs. 4 and 5)

I 2 cases (Nos. 1 and 15) the effect of triopron was compared with that of desiccated thyroid In both, the mean cholesterol fell by 50—100 mg/100 ml after desiccated thyroid was replaced by triopron. In both, however there was an increase in B. M R of between 0 and + 10 %. That the patients on desiccated thyroid did not have myxoedema was confirmed by the thyrotrophin stimulation test (10)

In another two (cases 13 and 19) the treatment was altered to l-triiodothyronine (tertroxin) after triopron had been discontinued for a couple of months. During the period without treatment the serum cholesterol level increased to the values found prior to the institution of

Table III Effect of l-triiodothyronine (tertroxin) upon the serum-cholesterol in 6 euthyroid patients with

Case	Sex	Age	Dose (μg)	Duration of treatm. (months)	Cholesterol (150—300 mg/100 ml)			
					a	b	c	d
1	F	64	20	4	339	− 94	− 44	13
			2—4 mg (triopron)	7	539	− 163	− 94	9
2	F	51	40	3	354	− 95	− 52	4
3	F	54	40	3	430	− 120	− 92	4
4	F	65	20−40	6	326	− 64	− 29	7
5	M	46	40	3	350	− 78	− 35	5
6	M	51	40	3	377	− 71	− 24	3

a = No treatment. b = Maximum alteration. c = Alteration from pre-treatment value to mean value determinations.

triopron. During tertroxin medication the cholesterol fell negligibly in one and rose in the other

An attempt was also made to treat another 2 patients (cases 7 and 22) with tertroxin but soon this had to be abandoned as now the patients developed anginal attacks.

The PBI increased in all cases of triopron medication to above the normal values except in two (cases 23 and 25) In 8 the T_3 uptake by the red cells was determined before and during triopron medication (cases 7 15 17 18, 19 20 23 26) It increased in 4 (cases 7 17 20 23) and remained unchanged in 4 In no case did it exceed the upper limit of normal.

Figs. 6 and 7 illustrate the course of the B M. R. cholesterol, and PBI in 2 patients (cases 1 and 3)

None of the 26 patients showed severe thyrotoxic signs at any time. One (case 3) had an increase in the pulse rate, but the B M. R. did not rise. On the other hand 18 patients lost weight. Six had annoying side effects, consisting in exacerbation of angina pectoris in 3 (cases 12 19 and 22) nervous symptoms in one (case 23) paroxysmal tachycardia in one (case 4) and palpitations in one (case 16) In all 6 the medication had to be discontinued.

In a total of 21 patients (table II cases 1 2 3 4 5 6 9 10 11 12, 13 16 17 18 19 20, 21 22 23 24 25) triopron was discontinued and the course of the serum cholesterol was followed during the subsequent months In 15 it increased within 2—8 weeks to the pre treatment values (cases 1 2 3 9 10 11 12 13 16, 18, 19 21 22 24 25) In 6 however this resulted in only a minor increase in cholesterol (cases 4 5 6 17 20 23) In these 6 patients the decline of cholesterol during the treatment had been moderate. The effect of triopron upon the cholesterol level appeared to persist as long as the medication was continued.

In order to ascertain the effect of l-triiodothyronine upon the serum cholesterol in euthyroid patients, 6 euthyroid subjects

hypercholesterolaemia

BMR (− 10 to + 10%)				PBI (3.0—8.0 μg%)				T_3-uptake (6.0—10.5%)	Pulse rate	Weight loss (kg)	Comments
	b		d	a	b		d				
+ 9	+ 9	− 2	7	8.0	− 6.6	− 3.8	10	8.4→8.3	66→84	− 2	Anginal attacks
+ 9	+ 7	− 7	6	8.0	+ 6.8	+ 0.4	5				
+ 9	+ 58	+ 17	4	7.4	− 4.1	− 2.0	3		62→72	+ 1	Anginal attacks
+ 2	+ 27	+ 11	4	4.7	− 4.3	− 1.6	4		66→64	− 3	Anginal attacks
− 7	+ 37	+ 14	8	5.3	+ 1.8	+ 1.0	2		63→67	− 3	
+ 4	+ 42	+ 14	4	6.5	− 3.9	− 2.5	3		61→61	0	+ Tremor + Nervousness
+ 5	+ 31	− 3	4	6.9	− 4.8	− 3.8	4		70→76	+ 3	

during treatment. d = Number of determinations during treatment. Before treatment there were three

with hypercholesterolaemia and coronary sclerosis were so treated. Case 1 also had xanthomatosis. Table III shows the result. The serum cholesterol level fell in two (cases 2 and 3) by between 50 and 100 mg/100 ml and in four (cases 1 4 5 and 6) by between 0 and 50 mg/100 ml. The B. M. R. rose in 4 (cases 2—5) by more than 10 %. In two (cases 1 and 6) there was a slight fall. No patient exhibited thyrotoxic symptoms although 3 lost weight (cases 1 3 and 4) Four developed annoying side effects, viz. anginal attacks and nervousness. The PBI fell in all but two cases (table II case 19 and table III case 4) a phenomenon well-known from the literature (Lerman et al. (16))

In case I the medication was changed from tertroxin to triopron, with the result that the serum cholesterol fell without the B. M. R. rising While in this case tertroxin had induced anginal attacks, triopron was much better tolerated.

Tables IV and V give a comparison of the results of treating euthyroid patients with triopron and with triiodothyronine Table IV shows the difference between the cholesterol and B. M. R. values before and the mean values during the treatment.

It is evident that the fall was on the whole greater in the triopron-treated than in the 8 tertroxin-treated patients (0.001 $< p <$ 0.01) (21.1 per cent of the values before treatment to 6.1 per cent) On the other hand, there was no definite difference between the increases in B. M. R. Tertroxin appeared more apt than triopron to give rise to annoying side effects, especially angina pectoris.

When comparing the maximum cholesterol decreases (table V) the findings are very much the same.

Discussion

In assessing the effects upon cholesterol metabolism, only total serum cholesterol was studied. No distinction was made between esterified and non-esterified cholesterol or between α- and β-cholesterol.

Table II Mean decline in cholesterol and mean increase in BMR in euthyroid patients (tables II and III) treated with triiodothyropropionic acid (triopron) and triiodothyronine (tertroxin)

	Mean decline in cholesterol in mg/100 ml						
	200–250	150–200	100–150	50–100	0–50	Increase	Mean decline (mg/100 ml)
Triopron		2	6	14	2	1	86.2 ± 13.5
Tertroxin				2	5	1	26.4 ± 48.5

	Mean increase in BMR in % of normal value							
	>50	40–50	30–40	20–30	10–20	0–10	−10–0	Mean increase (%)
Triopron				1	5	11	8	5.4
Tertroxin					4		4	6.4

Table I Maximum decline in cholesterol and maximum increase in BMR in euthyroid patients (tables II and III) treated with triiodothyropropionic acid (triopron) and triiodothyronine (tertroxin)

	Maximum decline in cholesterol in mg/100 ml						
	300–250	250–200	200–150	150–100	100–50	50–0	Increase
Triopron	3	3	13	5	1		
Tertroxin				1	5	1	1

	Maximum increase in BMR in % of normal value						
	>50	40–50	30–40	20–30	10–20	0–10	−10–0
Triopron		3	3	6	7	5	1
Tertroxin		1	3	1		1	2

In their study on the effects of PAS upon serum cholesterol Tygstrup et al (24) found the changes in total cholesterol to parallel in all essentials, the changes in esterified β-cholesterol

In the Lieberman Burchard reaction as used in the present analyses, cholesterol ester gives a somewhat stronger colour than free cholesterol When the reaction is employed for determining total cholesterol without preceding saponification one must know the ratio of free to esterified cholesterol in the sample in order to be able to calculate a true analytical result.

For each serum, Kingsley & Schaffert (15) carried out a determination of cholesterol ester after digitonin precipitation of the free cholesterol. Instead of such an individual correction for each serum, we calculated all the results on the basis of an assumption of 25 % free cholesterol. The errors which may have resulted from this procedure are negligible. At 40 % free cholesterol, the value will be 5 % too low. In a series like the present one in which none of the patients exhibited any signs of hepatic disease, the variation of free cholesterol is presumably even less marked. Moreover the analyses were used only for comparing the total cholesterol in the same patient at various junctures. The analytical uncertainty is moderate compared with the variations found in the cholesterol level.

Our investigations appear to show that triopron possesses a cholesterol-depressing effect which is rather varied, but does not give rise to essential thyrotoxic symptoms or signs. Five out of 25 euthyroid patients showed a rise of B. M. R. exceeding 10 %. The drug was well tolerated except in 6 patients, 3 of whom developed angina pectoris.

That triopron possesses a thyroid hormonal effect is beyond doubt. It exerts a completely compensatory action in myxoedema, as also demonstrated by Hill et al. (13) and Rawson et al. (19). Its effect upon the B. M. R. is stated to be only 5—10 % of that of thyroxine, whereas its effect upon the metamorphosis of tadpoles is 300 times greater (Rawson et al. (19)).

The cause of the marked increase in PBI in nearly all the triopron-treated patients is presumably due to an iodine effect. About 60 % of triopron is iodine, and on a dosage of 4—6 mg daily the patients receive more than 2 mg iodine daily which presumably explains the elevated PBI.

Table VI. Effect of thyrotrophic hormone (4 U.S.P. units) on the uptake of I^{131} by the thyroid gland in 4 euthyroid patients treated with triopron (table II)

Case	Before TSH		After TSH	
	% of dose administered		% of dose administered	
	4-hour uptake	24-hour uptake	4-hour uptake	24-hour uptake
7	9.1	6.4	34.4	56.0
12	8.5	9.7	37.2	58.0
15	6.2	0.8	10.0	11.4
25	5.9	5.6	8.5	17.5

Like other thyroid hormones triopron affects the function of the thyroid gland. Thus, in the dosage employed it inhibited the I^{131} uptake in the same way as does l-thyroxine and l-triiodothyronine. This has previously been described by Slater et al. (21) and Rawson et al. (19) who state that in this respect the drug is 60 % times as active as l-thyroxine. That this is not due to an iodine effect may be seen from the fact that the thyroid gland reacts to stimulation with thyrotrophic hormone, investigated in 4 cases by a technique which has been described previously (10) (table VI). If the iodine content of the drug were responsible for the inhibited uptake of I^{131} in the thyroid gland, there would be no reaction to thyrotrophic hormone (Bishopric et al. (6)).

It is evident from our studies that in non-myxoedematous patients too the thyroid hormone can depress the serum cholesterol without inducing any essential increase in the B. M. R. and the propionic acid analogue of triiodothyronine appears to afford a compound with a greater cholesterol-depressing effect in

relation to its metabolic effect than the current thyroid hormone preparations.

We feel that our investigations permit us to discuss the clinical applicability of triopron as a means of reducing the serum level of cholesterol. At this stage, however we shall not enter into the advisability of this step

The applicability of the thyroid hormones, and especially of triopron must be considered from two angles, partly actual side effects, and partly the effect upon the thyroid gland and its function The side effects have been described above and must be assessed in relation to the possible deleterious effect of the hypercholesterolaemia

Administration of thyroid hormone exerts an inhibitory effect upon the thyroid function via the thyrotrophic hormone (10) This effect presumably persists as long as the medication is continued It is unlikely that organic damage to the thyroid gland occurs, since after long term treatment with certain thyroid hormones, the thyrotrophin stimulation test has shown a normal function (10) The same indubitably applies also to triopron

Amongst other questions concerning the clinical applicability of thyroid hormones (e. g triopron) it is necessary to be certain that triopron has not similar or greater effect on the calcium balance as it is known in hyperthyroid states. Studies on this problem are now being performed in this department.

Summary

The effect of triiodothyropropionic acid (triopron) upon the serum cholesterol level, basal metabolic rate, and protein-bound iodine in the serum was investigated in 6 myxoedematous patients and 26 euthyroid patients suffering from coronary disease and hypercholesterolaemia.

Triopron exerted a fully compensatory effect on the myxoedema in the 6 myxoedematous patients.

In the 26 euthyroid patients with coronary disease the cholesterol fell to a varying extent. The mean decline was as follows In two cases 150—200 mg/100 ml in 6 cases 100—150 mg/100 ml in 13 cases 50—100 mg/100 ml in 2 cases 0—50 mg/100 ml and in 1 case a slight increase. The B M R. rose by more than 10 % in only 5 in whom the increase ranged from 10 % to 28 % The PBI rose in all but 2 patients to above normal values, presumably because of an iodine effect. Triopron was surprisingly well tolerated by patients with coronary disease. Only 6 developed angina pectoris, palpitations, and nervousness Eighteen lost a little weight. No definite thyrotoxic symptoms or signs were observed There was no relation between the pre-treatment cholesterol level and the extent of the decline.

In order to compare the effect with that of current thyroid hormone preparations 4 of the myxoedematous patients and 2 of the euthyroid patients were treated also with desiccated thyroid. The serum cholesterol level was considerably lower in 4 of these patients during triopron medication. In 3 however the B M R. was on the whole 8—13 % higher

Eight patients were treated with l-triiodothyronine which made the serum cholesterol fall by 50—100 mg/100 ml in 2 0—50 mg/100 ml in 5 and increase in one The B M R rose by more than 10 % in 4 cases. Five patients developed anginal attacks. Thus, on an average the serum cholesterol fell more in the triopron-treated cases, while there was no definite difference in the increase in B. M R. in the two groups.

It is concluded that triopron can, to a varying extent, lower the serum cholesterol in euthyroid patients with hypercholesterolaemia without essentially affecting the basal metabolic rate.

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Myocardial Infarction in the Younger Age Groups

II. Follow-up Observations with Special Reference to Capacity for Work

By

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The case records of patients with their first myocardial infarction below the age of 55 treated at one of the medical clinics of the general hospital of Göteborg from 1948 up to the end of 1957 have been reviewed. The result of this study has been reported earlier (7) The present communication is concerned with a follow-up study of the survivors of this series. In this section we present the results of a general clinical examination and electrocardiographic data. A further report will deal with the results of exercise tolerance tests in relation to the electrocardiographic response during and after exercise and to physical working capacity

Material

The original material consisted of 318 cases of myocardial infarction. At the time of the follow-up 195 patients were alive Of these 155 cases could be re-examined (79 per cent) Of the remaining 40 23 had moved from the town and 17 could not be examined for various other reasons or because they refused examination.

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The age and sex distribution of the re-examined patients is given in table I. The mean age of the whole series was 48 years. The interval between the acute illness and the follow-up is seen in table II. The verage time was 3 years and 5 months. Table III shows the clinical and electrocardiographic criteria of the material at the time of hospital treatment.

Before the re-examination 32 patients (21 per cent) had another infarction and 6 patients (4 per cent) had two additional infarctions. Predisposing diseases were found in 71 patients. diabetes mellitus in 5 hypertension in 61 (43 per cent) essential familial hypercholesterolaemia in 3 and hypothyroidism and valvular heart disease in one each.

Methods

Each patient was asked to ttend for re examination. The follow-up examinations were performed in the morning t the hospital. The medical history was checked as to heredity previous diseases, eating, smoking and alcohol habits, symptoms of angina pectoris and heart failure medication and capacity for work. Then physical examination was performed and blood sample was obtained for analysis of serum cholesterol. In some cases an X-ray of the heart and chest was taken and the

Diet, smoking and alcohol habits

Fat food was avoided by 54 (35 per cent) and 15 (10 per cent) seemed to eat exceptionally fat food as compared with ordinary dietary standards in Sweden (35 to 40 per cent of the calories are supplied as fat in this country). Sixty-four patients were moderate smokers (41 per cent) and only seven smoked more than 20 cigarettes per day (5 per cent). There was a moderate alcohol consumption in 62 cases (40 per cent) and 11 cases (7 per cent) drank more than 2 litres of hard liquor per month.

Heart failure

There were symptoms and signs of congestive heart failure in 73 (43 per cent) cases. In 17 cases there was only dyspnoea on exertion or nocturnal dyspnoea. In 13 cases oedema of the ankles occurred in the evening or nocturia was present. In 43 cases there was a combination of these symptoms. Of the patients with symptoms of heart failure 95 per cent used digitalis preparation and among those without evidence of heart failure 18 per cent used this drug.

Angina pectoris

Angina of effort was recorded by 81 patients (51 per cent) and chest pain without relationship to exertion in 21 (14 per cent). Fifty-seven per cent of the patients with angina of effort used some nitrite medication and of those without any pain 15 per cent used nitrites.

X-ray examination

X-ray examinations of the heart and chest were obtained at random in 34 cases. In 18 patients the cardiac outlines were enlarged and 2 cases presented signs of pulmonary congestion. In 5 cases abnormal pulsations of the cardiac outlines could be detected, 4 of these cases had also cardiac enlargement. The upper limit of heart volume for men was put at 400 ml/m body surface area and at 350 ml/m for women measured according to Jonsell (5). The patients showing pulmonary congestion had also clinical symptoms of heart failure.

Serum cholesterol

Serum cholesterol was determined in 147 cases. The results are evident from table IV and have been discussed elsewhere (2).

Resettlement and capacity for work

At re-examination of the total material of 318 cases 39 per cent had died and about 34 per cent had regained full working capacity. There were 3 patients who were unable to work even before the infarction. Among the remaining 150 patients 63 (42 per cent) had returned to work within 4 months. 64 (43 per cent) had returned to work later than 4 months after the acute episode but within 1 year. Nine patients (6 per cent) had returned to work even though they had not worked for more than 1 year after the illness. Fourteen patients (9 per cent) did not return to work.

At the time of the follow-up examination (average time 3 years, 5 months) after the acute illness 104 (69 per cent) of the 150 cases with unlimited pre-infarction work status had full capacity for work although 22 of these had changed to a physically less demanding work. There was diminished capacity for work in 27 (18 per cent) cases and 19 (13 per cent) were unable to work.

Table V gives a work classification of

Table I Age and sex distribution

	30–35 years	35–40 years	40–45 years	45–50 years	50–55 years	Total
Men	1	4	25	37	62	129 (83%)
Women	0	0	4	6	16	26 (17%)
Total	1	4	29	43	78	155

Table II Time interval between myocardial infarction and follow-up

Years	No. of cases
0.5–1	1
1–2	36
2–3	32
3–4	15
4–5	7
5–6	17
6–7	13
7–8	20
8–9	11
9–10	3
Total	155

Table III Combination of clinical and electrocardiographic criteria

	Q ± ST elevation	ST + T alteration	No ECG signs	Total
Typical symptoms and signs	102	26	3	131
Typical symptoms	10	8		18
Typical signs	1	3		4
No clinical evidence	1	1		2
Total	114	38	3	155

heart volume calculated and in some cases electrokymography was performed. In all patients an electrocardiogram was taken at rest. The majority of patients also performed an exercise tolerance test on a bicycle ergometer. The exercise tolerance test was excluded if there were markedly pathological electrocardiograms at rest, history of a second myocardial infarction, or recent attacks of angina pectoris.

Table IV Serum cholesterol values at follow-up. In eight patients there was no serum cholesterol value available

Cholesterol mg/100 ml	No. of cases
< 200	5 (3%)
200–250	21 (14)
250–300	55 (3 %)
> 300	66 (45%)
Total	147

The electrocardiographic review of records obtained during the acute illness is based on tracings with four leads (I, II, III and IVa) in two thirds of the cases. In the remaining patients three additional chest leads (CR_2, CR and Nehb's differential lead J) had been recorded. In all instances serial tracings of at least 3 electrocardiograms were taken and in most cases 7 tracings were available. At re-examination the electrocardiogram consisted of th three standard extremity leads I, II and III, three unipolar extremity leads, aVR, aVL and aVF and 5 precordial R-leads from the positions C_1, C_2, C_4, C_5 and C_7.

Results

Hereditary factors

In 32 per cent of the patients there was a history indicating incidence of cardiovascular and cerebrovascular disease among parents or brothers and sisters.

In table VI and VII the incidence of angina pectoris and heart failure is listed in relation to capacity for work at re-examination among 150 cases with full working capacity prior to infarction.

From table VI can be seen that there are more cases who were unable to work among the patients suffering from effort angina than among those without chest pain ($p < 0.05$) Table VII shows that there were 84 per cent with full working capacity of those without heart failure but only 52 per cent with full working capacity among the cases with evidence of congestive heart failure. In the category of patients without heart failure there was a significantly lower incidence of patients who were unable to work compared with the group of patients with congestive heart failure ($p < 0.001$)

Electrocardiogram at re-examination

In table VIII are listed the electrocardiographic findings during the hospital stay and at re-examination. Of the 152 cases with abnormal electrocardiographic signs during the acute stage of the illness there were 25 cases (16 per cent) with a completely normal ECG at re-examination. In 9 patients an arrhythmia was the only abnormal electrocardiographic sign. Forty two cases (28 per cent) had isolated pathological Q waves and 52 (34 pr cent) had abnormal Q waves in combination with ST and T alterations. 24 cases (10 per cent) had only ST and T changes.

In the group of patients who originally presented definit electrocardiographic signs of infarction (Q ± ST elevation) during the acut stage of the illness there were 12 cases (10 per cent) with a completely normal ECG at re-examination. Among the patients with suggestive electrocardiographic signs of infarction (ST

Table IX Electrocardiographic signs at re-examination in relation to angina pectoris

ECG at re-examination	No chest pain	Uncharacteristic pain	Angina pectoris	Total
Q ± ST + T alterations	31	13	51	95
ST + T alterations	10	4	14	28
Arrhythmia	2	–	3	5
Normal	10	4	13	27
Total	63	21	81	155

Table X Electrocardiographic signs during hospital stay in relation to capacity for work. Five patients who were unable to work already prior to infarction, one case with normal electrocardiogram during the course of illness and two cases with "old" Q waves prior to infarction have been omitted

ECG during hospital stay	Capacity for work			Total
	Ordinary	Limited	Unable to work	
Q ± ST elevation	86	15	9	110
ST + T alterations	17	10	10	37
Total	103	25	19	147

Table XI Electrocardiographic signs at re-examination in relation to capacity for work. Five patient who were unable to work already prior to infarction are excluded

ECG at re-examination	Capacity for work			Total
	Ordinary	Limited	Unable to work	
Q	20	4	1	25
Q ± ST + T alterations	47	12	9	68
ST + T alterations	15	5	7	27
Arrhythmia	2	2	–	4
Normal	20	4	2	26
Total	104	27	19	150

Table I. Classification of work in arbitrary units as to physical and intellectual capabilities bef[illegible] infarction and subsequent employment afterwards. Five patients who were unable to work already prior to infarction are excluded

Employment status	Work classification: Physical labour +++	++	+	Intellectual work +++	++	+
Unable to work	6	6	7	2	3	[illegible]
Restricted	5	5	17	3	6	[illegible]
Full work (A)	6	8	8	4	4	[illegible]
Full work (B)	5	38	39	22	22	[illegible]
Total	22	57	71	31	35	84
Summary	150			150		

(A) = return to lighter work. (B) = return to same work.

Table VI. Angina pectoris and capacity for work. Five patients who were unable to work already prior to infarction are excluded

Angina pectoris	Capacity for work: Ordinary	Limited	Unable to work	Total
No chest pain	46	3	4	53
Uncharacteristic pain	14	2	3	19
Anginal	44	22	12	78
Total	104	27	19	150

Table VII. Heart failure and capacity for work. Five patients who were unable to work already prior to infarction are excluded

Heart failure	Capacity for work: Ordinary unlimited	Limited	Unable to work	Tot[illegible]
No heart failure	68	10	3	81
Heart failure	36	17	16	69
Total	104	27	19	150

Table VIII. Electrocardiographic signs during hospital stay and at re-examination. One case with a normal electrocardiogram during the course of illness and at re-examination and t[illegible] cases with "old" Q waves prior to infarction have been omitted

ECG during hospital stay	ECG at re-examination: Q ± ST+T alterations	ST + T alterations	Arrhythmia	Normal	Total
Q ± ST elevation	85	10	7	12	114
ST + T alterations	9	14	2	13	38
Total	94	24	9	25	152

the patients prior to infarction in relation to employment status at the time of the follow-up. From this is evident that there is a higher rate of resettlement to the original occupation among the cases with the highest degree of intellectual work, compared with the ordinary worker with heavy physical labour.

In table VI and VII the incidence of angina pectoris and heart failure is listed in relation to capacity for work at re-examination among 150 cases with full working capacity prior to infarction.

From table VI can be seen that there are more cases who were unable to work among the patients suffering from effort angina than among those without chest pain ($p < 0.05$). Table VII shows that there were 84 per cent with full working capacity of those without heart failure but only 32 per cent with full working capacity among the cases with evidence of congestive heart failure. In the category of patients without heart failure there was a significantly lower incidence of patients who were unable to work compared with the group of patients with congestive heart failure ($p < 0.001$).

Electrocardiogram at re-examination

In table VIII are listed the electrocardiographic findings during the hospital stay and at re-examination. Of the 152 cases with abnormal electrocardiographic signs during the acute stage of the illness there were 25 cases (16 per cent) with a completely normal ECG at re-examination. In 9 patients an arrhythmia was the only abnormal electrocardiographic sign. Forty-two cases (28 per cent) had isolated pathological Q waves and 52 (34 per cent) had abnormal Q waves in combination with ST and T alterations. 24 cases (10 per cent) had only ST and T changes.

In the group of patients who originally presented definite electrocardiographic signs of infarction ($Q \pm ST$ elevation) during the acute stage of the illness there were 12 cases (10 per cent) with a completely normal ECG at re-examination. Among the patients with suggestive electrocardiographic signs of infarction (ST

Table IX. Electrocardiographic signs at re-examination in relation to angina pectoris

ECG at re-examination	No chest pain	Uncharacteristic pain	Angina pectoris	Total
$Q \pm ST + T$ alterations	31	13	51	95
$ST + T$ alterations	10	4	14	28
Arrhythmia	2	—	3	5
Normal	10	4	13	27
Total	63	21	81	155

Table X. Electrocardiographic signs during hospital stay in relation to capacity for work. Five patients who were unable to work already prior to infarction, one case with normal electrocardiogram during the course of illness and two cases with "old" Q waves prior to infarction have been omitted

ECG during hospital stay	Capacity for work			Total
	Ordinary	Limited	Unable to work	
$Q \pm ST$ elevation	86	15	9	110
$ST + T$ alterations	17	10	10	37
Total	103	25	19	147

Table XI. Electrocardiographic signs at re-examination in relation to capacity for work. Five patients who were unable to work already prior to infarction are excluded

ECG at re-examination	Capacity for work			Total
	Ordinary	Limited	Unable to work	
Q	20	4	1	25
$Q + ST + T$ alterations	47	12	9	68
$ST + T$ alterations	15	5	7	27
Arrhythmia	2	2	—	4
Normal	20	4	2	26
Total	104	27	19	150

and T alterations) there was complete restitution in 34 per cent. In the same group there were now 9 (24 per cent) with cases abnormal Q waves that had been absent before. Four of these were known to have had new infarctions. There was thus a higher rate of electrocardiographic restitution in cases with ST and T wave changes compared with those who had abnormal Q waves and ST elevation ($P < 0.01$)

There was no significant relationship between extent of electrocardiographic signs during the hospital stay and the incidence of effort angina as evident from follow up inquiries. Table IX shows the electrocardiographic findings at re-examination in relation to effort angina. There is a higher incidence (63 per cent) of major electrocardiographic abnormalities in the category of patients with angina of effort compared with those without chest pain (49 per cent) This difference is, however not statistically significant when compared with the incidence of normal tracings in these two groups.

No correlation could be found between the electrocardiographic findings during the acute stage of myocardial infarction or at re-examination and the incidence of congestive heart failure

In table X and XI the electrocardiographic findings during hospital stay and at re-examination are listed in relation to assessed capacity for work. Changes of ST and T wave seem to have a less favourable implication on a patients capacity for work than abnormal Q waves. This applies both to the electrocardiographic findings during the acute period and to those at re-examination. ($P < 0.001$) Isolated Q wave abnormalities were connected with the same incidence of ordinary capacity for work as normalized electrocardiograms at the follow up

Discussion

Hereditary factors

There are numerous reports stressing the familial incidence of cardiovascular disease e. g Billings et. al (1) found a family history of arteriosclerotic heart disease in 45 per cent of their patients with coronary heart disease. Gertler and White (3) could show significantly higher death rates in cardiovascular disease in fathers and siblings of young patients with myocardial infarction than in fathers and siblings of a control group In the present series the incidence of cerebro-vascular and cardiovascular disease among parents and siblings of our patients was 32 per cent.

Diet smoking and alcohol habits

Many patients of this series avoided fat food after having experienced myocardial infarction. This factor should if anything, have contributed to lower the serum cholesterol values. In spite of this the cholesterol values are high when compared with normal standards (6) The incidence of 5 per cent of heavy smokers as well as 7 per cent with a rather high alcohol consumption is probably in conformity with average figures of a corresponding part of the Swedish population. Gertler and White (3) found no difference between their patients and a control group in these respects

Heart failure

Mintz and Katz (10) reported 121 instances of congestive heart failure during hospital stay after myocardial infarction. The mortality in this group was 42 per cent as compared with 22 per cent of the whole series. The poor prognosis in cases who develop heart failure early after myocardial infarction has also been pointed

out by White et al. (15) Of 18 such patients only two were alive after 5 years. In the follow up study of Iisalo et al. (4) there were about 41 per cent with evidence of heart failure among the survivors six months to five years after myocardial infarction. Our figure of 43 per cent agrees well with their findings. Of these one fifth had symptoms or signs of isolated right heart failure, one fifth of left heart failure only and the remaining cases had evidence of combined right and left heart failure. Nearly all the patients with heart failure used digitalis preparations and of those without 18 per cents received digitalis therapy In the latter group earlier symptoms of heart failure may have disappeared at re-examination. The ultimate prognosis in those patients who gradually developed heart failure some years after the initial infarction cannot yet be assessed but is more favourable than for those who developed heart failure during the first month.

Angina of effort

Mintz and Katz (10) had 69 per cent patients with angina pectoris during the hospital stay after myocardial infarction. The mortality in that group was 39 per cent as compared with 22 per cent in the whole series. White et al. (15) reported 18 patients with angina pectoris one month after myocardial infarction. Four of them were alive 5 years later Iisalo et al. (4) found effort angina of varying severity in 6 per cent of their series and we had effort angina in 52 per cent and uncharacteristic chest pain in another 14 per cent.

Serum cholesterol

There were remarkably many cases with high serum cholesterol values when compared with normal material and with the mean values during the stay in hospital These findings have been discussed in more detail elsewhere (2) In 33 cases there were sufficient data for comparing the cholesterol values on the first to the third day of illness with those on the fourth to the thirteenth day and with the values at re-examination. On the first occasion the mean value was 260 mg per 100 ml, on the second 243 and on the third 300 During the hospital stay there were 17 per cent of the cases with mean cholesterol values over 300 mg per 100 ml and at re-examination 46 per cent of the examined patients had values over 300 The cholesterol values taken during hospital stay after a myocardial infarction do not show the true level in the particular patients as pointed out by Welin (14)

The cholesterol values at re-examination in the patients without hypertension diabetes, familial essential hypercholesterolaemia, hypothyroidism, organic heart disease or positive Wassermann reaction were significantly higher than the values of normals from the same population in the age groups of 40 to 49 years and 50 to 59 years (2 6) The patients with hypertension as predisposing disease had cholesterol values that were between the values of the normals and those of the patients without special predisposing disease.

Resettlement and capacity for work

Iisalo et al. reported that at the follow up examination, 6 months to 5 years after myocardial infarction 61 per cent had regained full working capacity Of these 6 per cent had changed to a physically less demanding work. Master et al. (8) investigated cases who had survived at least one year and found that 73 per cent had returned to activity Morris and Morris (11) found that 37 per cent of those who had returned to employment after their first

myocardial infarction were still working more than 5 years later In our series 91 per cent of the survivors finally returned to some form of gainful activity

Thus, a very high proportion of patients surviving myocardial infarction are able to return to activity There is, however a close connection between return to activity and the type of employment or the possibility of getting physically less demanding work. In our series of 22 workers who had performed hard physical labour only 5 had been able to continue in the same employment. Another 6 in the same group regained full working capacity when they found less strenuous work. In the group of cases with employment of minor physical requirements there were 73 per cent with full working capacity Patients with an occupation implying less physical labour are more apt to return to their original work. All cases with an occupation implying heavy manual labour should have a special evaluation of their functional capacity in order to determine the most suitable type of employment. It is desirable to start rehabilitation during the period of convalescence. The physician social worker and vocational counselor must co-operate in order to achieve selective placement of the patient

That there were fewer patients who regained full working capacity in the category of patient with symptons or signs of heart failure or angina pectoris seems quite reasonable but the figure of about 50 per cent in these two groups must be considered a high value in view of the presence of disabling symptoms.

Electrocardiography

It seems rather remarkable to find such a high percentage (16 per cent) of normalized electrocardiograms at the time of re-examination after an accute episode of myocardial infarction with electrocardiographic alterations during the acute period Even pathological Q waves, the hallmarks of myocardial damage, disappeared in a high proportion of cases (10 per cent) The still higher incidence of normalized electrocardiograms (34 per cent) in the group with suggestive electrocardiograph signs (ST and T alterations) on the other hand is not surprising Mills et al. (12) reported complete electrocardiographic restitution in 9 per cent. Iisalo et al (4) had 22 per cent with completely normalized electrocardiograms.

There was an equal incidence of cases with angina pectoris after the infarction among those who had definite signs of infarction during the hospital stay and those who only presented suggestive signs. There was moreover no consistent trend in the distribution of angina pectoris in different groups of electrocardiographic patterns at re-examination. The electrocardiogram at rest was thus of no value in predicting the development of angina pectoris in this material.

When considering capacity for work the incidence of abnormal Q waves was not connected with a higher rate of disability than normal electrocardiograms at re-examination. The groups consisting of cases with only ST and T alterations at re-examination had the lowest percentage of cases with full working capacity The group of cases with pathological Q waves together with ST and T alterations presented intermediate values.

Summary

The results of a follow up study of 155 survivors of a series of 318 cases with myocardial infarction are presented The patients under consideration were below the age of 55 when they experienced their

first myocardial infarction, thus belonging to the working population. The following conclusions may be made

1. A family history of cardiovascular and cerebrovascular disease was present in 52 per cent.

2. There was evidence of congestive heart failure in 43 per cent and of angina pectoris in 52 per cent.

3. Blood pressures above 160 mm Hg systolic or above 90 mm Hg diastolic were found in 45 per cent of the patients, diabetes mellitus in 5 per cent and essential familial hypercholesterolaemia in 2 per cent. Twenty-two per cent had cholesterol values over 300 mg per 100 ml but none of the above mentioned predisposing diseases. In the remaining 25 per cent of the patients there was not any known predisposing factor.

4. Sixty-nine per cent had regained full working capacity, 18 per cent had diminished capacity for work and 13 per cent were unable to work. There was a lower rate of resettlement in the original employment in the category of cases with heavy physical labour as compared with the "intellectual" workers. In this connection the need for selective placement and rehabilitation is stressed. — Angina pectoris and congestive heart failure adversely affected return to work.

5. Complete restitution of the electrocardiogram occurred in 16 per cent. There was a higher rate of electrocardiographic restitution among the cases who originally presented ST and T alterations (34 per cent) as compared with those having abnormal Q waves (10 per cent). — The electrocardiogram at rest was no of value in predicting angina pectoris or congestive heart failure in this material. ST and T wave changes seem to have a less favourable implication on patient's capacity for work than abnormal Q waves.

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Arterial Hypertension of Complicated Origin

By

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General Introduction

When confronted by a case of arterial hypertension it is of paramount importance to study its origin, since several symptomatic types of arterial hypertension are liable to benefit from surgical intervention. Such may for instance, be the case in

1 Coarctation of the aorta
2. Endocrine causes, mainly suprarenal (Conn, Cushing, pheochromocytoma)
3 Renal causes, particularly the one sided lesions
4 Increased intracranial pressure, as for instance epidural hematomas but also subdural hematomas and certain neoplasms.

On the whole, this symptomatic group of hypertension is apt to exceed by far the 10 % originally allotted to it. With regard to the one-sided renal lesions, such an affection is to be considered particularly if the following requirements are fulfilled

1 Lack of familiar occurrence of hypertension
2 Young age
3 Different size of the two kidneys
4 Hypogenital condition
5. Rapid progression of malignant hypertension.

As for the character of the lesion involved, this is mainly of two types

1 Affection of the renal arteries (partial block, aneurysms etc.)
2 Malformations of the kidney with or without pyelonephritis.

The methods of studying the presence of a one-sided renal lesion are mainly roentgenological, and only secondarily a study of the functional behaviour of each kidney separately

The roentgenological methods are particularly

1 A survey of the kidneys and their size without any contrast. This method is particularly valuable if renal insufficiency is present.

2. Urography with study not only of the pelvic structures, the calices etc. but also of the concentration and excretion of the contrast by the renal parenchyma. This method may be combined with pyelography from below

3 Arteriography from the abdominal aorta, by means of a Seldinger or Ödman

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catheter which is being passed from below (the femoral artery) or if necessary from above (the subclavian) to the approximate level of the superior mesenteric artery Direct puncture of the aorta or selective angiography after passing a catheter into the renal artery may be used in selected cases These methods not only give a clear view of the origin the size and the course of the renal artery and its branches but also as nephrography of the behaviour of the parenchymatous tissue

Catheters may be passed up into both ureters and the differential function of each kidney studied separately This method may be combined with catheterization of the renal veins so as to allow a determination of the clearance of each kidney However valuable these methods may be they tend to be more circumstantial than the roentgenological ones and it has been pointed out (by Poutasse and Page) that the evidence obtained may be rather contradictory and different if a one sided pyelonephritis is present as compared with the conditions recorded if a one sided lesion of the renal artery should be the cause.

Determination of the radioactive scintillations from both kidneys on excretion of some compound containing I^{131} may be considered as a combination of roentgenology and physiology when the approach to a one-sided kidney lesion is concerned So far we have lacked facilities for this method, although our colleagues in Stockholm have kindly carried out one analysis along these lines in one case of ours. The analysis was hardly conclusive

Material

Out of several instances of arterial hypertension in young people, the following two cases appear particularly instructive.

Case 1

A man, aged 20 who had to the best of his knowledge previously been healthy was subjected to a routine examination for employment. A high blood pressure was found and the man was referred to us for further analysis. His general condition was found to be good. There were three vascular nevi in the lumbar region of the skin. The blood pressure was 260/140 in his arms, 250/120—140 in his legs. Eyegrounds showed somewhat narrowed arteries and one minute aneurysm in the right eye. A systolic murmur was heard over the heart, but it had its maximum when auscultated from the abdomen a little to the left of the midline, exactly where the left renal artery was to be found. The tentative diagnosis was accordingly an aneurysm of the renal artery with subsequent hypertension. Since however his blood pressure was higher in his arms than in his legs, there was also to be considered some kind of coarctation. In order to find out whether this was the case and if so whether the coarctation could possibly be found in the abdominal aorta aortography was performed by one of us (H L.) the catheter being passed in through the right brachial artery which was strikingly superficial and a bit tortuous (fig 1) The following positive observations were made

1 Descending thoracic aorta narrow

2 Almost walnut-si ed aneurysm at origin of the left renal artery

3 A similar aneurysm at the origin of the celiac artery

4 Occlusion of the proximal part of the right renal artery A pea-sized aneurysm in the renal hilum. The right kidney supplied through collaterals

5 Narrowed origin of left renal artery

6 Inferior mesenteric artery enlarged, taking over a lot of the supply ordinarily furnished by the superior mesenteric artery

Two days after this investigation the young man had a subarachnoid hemorrhage which was repeated 16 days later and eventually killed him 4 days afterwards. The necropsy confirmed the observations made by aortography and added two more features a hazelnut-sized ruptured aneurysm in th anterior communicating artery and the presence of an infantile type of coarctation, at its usual site immediately below the origin of the left subclavian artery It allowed a pencil to be passed through.

In this case, then, we had obviously at least two features favouring the development of arterial hypertension

1 the coarctation and

2. the interference with the origin of the renal arteries.

If the multiple aneurysms in this case are considered — in the right eye, in the subarachnoid space (anterior communicating artery) in the abdominal arteries (the origin of the celiac artery and the origin of the left renal artery) and in a right-sided renal arterial branch — it is evident that a certain congenital inferiority of the arterial system has to be presumed. This interpretation seems to be amply corroborated by the hypoplasia of the descending aorta. It is rather startling to note that this man had done his military service without difficulties, serving in the infantery A review of the question of aneurysms of the renal artery will be found in the paper of Anderson (1)

Case

A man, aged almost 18, was referred to the Medical Clinic on April 1st 1961 When aged 13 routine examination in school is said to have revealed systolic murmur all over the heart and some degree of arterial hypertension (165 110—170 85) Otherwise he had been previously healthy with the exception of the infections of childhood and allergy (vomiting) towards fish-balls. When 14 surgical exploration revealed the presence of hypoplasia of the aorta from the level of few cm distal to the origin of the left subclavian artery down to the diaphragm T ompensate the internal mammary arteries were wide and serpiginous. Lumbar aortography revealed a reduced caliber of the abdominal aorta from the level of the diaphragm to the second lumbar vertebra, particularly pronounced at the level of the origin of the superior mesenteric and the renal arteries Collateral supply was established by means of the lower intercostal arteries and the arteries in the abdominal wall as well as between the inferior mesenteric and the superior mesenteric arteries These observations in 1957 were corroborated in 1961 (fig 2) when it was noted that the left renal artery had its origin above the maximal reduction of the abdominal aorta and that its caliber was fairly normal and its filling with contrast good. The superior mesenteric artery had its caliber very much reduced the first 4—5 mm of its course afterwards being normal, although the contrast only moved sluggishly The right renal artery was severely reduced in caliber for the first 7—8 mm from its origin from the aorta where it regained a normal size. The contrast was pronounced and strong in the left kidney very thin in the right. A walnut-sized aneurysm was found in the tenth intercostal artery on the right side. On March 10th a by-pass operation was performed in the department of thoracic surgery (Viking Olov Björk). A graft was made to pass the abdominal coarctation (from 10 cm above the diaphragm to 5 cm below the origin of the left renal artery) The right renal artery was connected by graft with the abdominal aorta below the lower end of the other graft. On April 1st the output of urine suddenly rose from about 2,000/24 hrs to 5 liters whereupon he was transferred to the medical clinic. The boy was found to be slender he had no cyanosis nor edema but was slightly dyspnoic. The blood pressure was 235 170 in the right arm, left arm 230 165, right leg 205/155, left leg 205/160. Eyegrounds some thin exudates. Pulse rate 125. Heart size 550 ml/m² with some relative enlargement of left ventricle Creatinine 0 6 mg endogenous creatinine clearance 68 ml. Antistaphylococcal titer 3.6 (= increased) His legs which had been cold and moist before the operation now were warm and comfortable. A postoperative angiogram showed good function of the graf passing the aortic coarctation but no passage through the graft to the right kidney A pea-sized aneurysm was found in a suprarenal artery on the lef side (not visible on previous angiograms)

In this case there can hardly be any doubt that whereas the result of the by passing of the abdominal coarctation was good in itself the right kidney still functions as a Hartwich-Goldblatt kidney and ought to be removed. The case markedly resembles that recently reported by Stokes and collaborators (12) As a matter of fact nephrectomy has recently



Fig 1 a

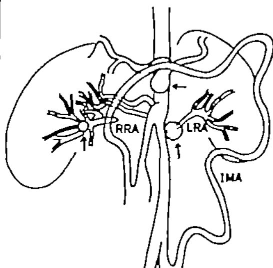


Fig 1 b

Fig 1 a and b Case 1 Narrowing of the aorta and the origin of the left renal artery (LRA) Occlusion of the proximal part (3 cm) of the right renal artery (RRA) Aneurysms (⟶) at the origin of the celiac artery and the left renal artery and in the right renal hilum A plexus of collaterals at the right border of the ertebral column supplying the right kidney The inferior mesentric artery (IMA) enlarged taki g over a lot of th supply ordinarily furnished by the superior mesenteric artery



Fig 2 a

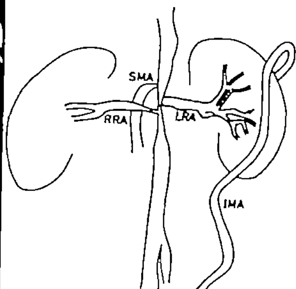


Fig 2 b

Fig 2 a and b. Case 2. Narrowing of the aorta with maximum just below the left renal artery (LRA) which has normal width also at its origin. The superior mesenteri artery (SMA) narrowed at the origin as the right renal artery (RRA) The left kidney has good ascular supply on th right side poor and retarded renal circulation. The intercostal arteries are widened. A prevertebral collateral plexus is isible at the height of coarctation The inferior mesenteric artery (IMA) enlarged as in case 1 Th aneurysms mentioned earlier in the paper were isible only on lat angiograms in the series. They are thus not demonstrable on the angiogram reproduced.

d

f

Fig. 1 d, f. Post-mortem observations. In ( ) the arterial system is prepared and compared with the caliber of the inferior vena ca adjoined. I (d) the infantile coarctation is to be seen, an the aneurysm of the left renal artery and in (f) probe introduced into the narrow origin of the right renal artery.

Erik Ask Upmark and Hrabia Lorés. Arterial Hypertension of Complicated Origin.



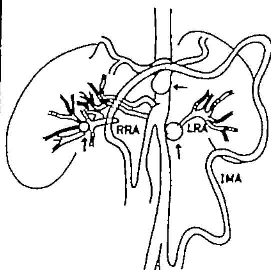


Fig 1 a

Fig 1 b

Fig 1 a and b. Case 1 Narrowing of the aorta and the origin of the left renal artery (LRA) Occlusion of th proximal part (3 cm) of the right renal artery (RRA) Aneurysms (⟶) at the origin of the celiac artery and the left renal artery and in the right renal hilum A plexus of collaterals at the right border of the vert bral col mn supplyi g the right kidney The inferior mesentric artery (IMA) enlarged taking over a lot of th supply ordinarily furnished by th superior mesenteric artery



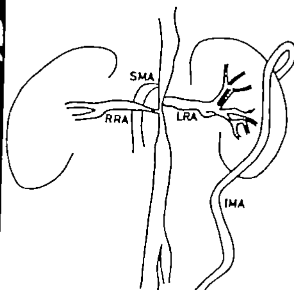


Fig 2 a

Fig 2 b

Fig 2 a and b. Case 2. Narrowing of the orta w th maximum just below the left renal artery (LRA) which has normal width also at ts origin. The superior mesenteric artery (SMA) narrowed t the origin as the right renal artery (RRA) The left kidney has good vascular supply on the right side poor and retarded renal circulation The intercostal arteries are widened. A prevertebral collateral plexus is visible at th height of coarctation. The inferior mesenteri artery (IMA) enlarged as in case 1 The aneurysms mentioned earlier in the paper were isible only on late angiograms in the series. They are thus not demonstrable on the angiogram reproduced.

been carried out with ensuing drop of the high blood pressure to normal level. The case also turned out to be of interest because of the large amounts of aldosterone, excreted whilst he still was in possession of the right kidney. Levels up to 150 mg in the urine of 24 hours were recorded. This extremely pronounced secondary aldosteronism immediately subsided on removal of the kidney. It will be dealt with in another paper.

Comment and discussion

In both these instances a striking feature was the reduced size of the descending aorta. Attention to the reality of aortic hypoplasia has already been called in another paper from our department, where some references are to be found (11). Other important contributions to this topic have been rendered by Apelt (2) and by Diamant (9). Quoting the pioneer observer in this field, Morgagni, Diamant makes the interesting remark: "In zwei anderen Fällen fand er (i. e. Morgagni) bei jungen Männern den ganzen Stamm der Aorta zu eng, *hierbei hatte sich das Venensystem dermassen erweitert dass Morgagni sagen konnte noch nie eine weitere Hohlvene gesehen zu haben*" (italics ours). This observation, incidentally, tallies well with the remarkable caliber of the inferior vena cava in case I as observed post mortem.

As maintained already by Apelt the vast majority of cases with hypoplastic aorta (aorta angusta) is apt to present enlargement of the heart. This was found also in the case earlier reported from this clinic (11). The enlargement is apt to involve both ventricles and it seems reasonable to assume that the blood pressure has been increased. In the observation described by Lander no increase in blood pressure was found but in this case it dealt with a severe cardiac insufficiency where the blood pressure level can hardly be considered significant. One may well ask whether the hypertension is related only to the reduced size of the arterial system (reduction of the "Windkessel") or whether secondary alterations of the wall may encroach upon the origin of the renal arteries. Both possibilities may be present. In case IX of Diamant it is emphasized that one of the kidneys was small and the uterus child-sized only. It is of some interest to recollect the assumption of Virchow on a relationship between a deficient development of the vascular system and deficient development of the genital structures (cf. also the hypoplasia of the ovaries sometimes observed in coarctation of the aorta, with ensuing sterility).

On the other hand one may well ask about the formal genesis of abdominal aortic coarctation. When the usual type of coarctation is present there can be little doubt about its connection with the branchial structures: it represents, as a matter of fact, a deficient involution of that part of the left aortic arch which is apt to disappear in connection with the establishment of a right-sided aortic arch (3). Moreover the hydrodynamic conditions prevailing during the intrauterine life with the privileged nutrition of the head and the upper part of the body may be called upon as additional evidence. As for the abdominal coarctation with its fate-loaded predilection for the level of the renal arteries it is difficult to furnish an entirely satisfactory explanation. It should be recalled, however, that the renal arteries during embryological development are climbing along the abdominal aorta in cranial direction (7) and it is entirely possible that this migration may entail irregularities at their origin from the ab-

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Hemodynamic Effects of Methyldopa (Aldomet®) at Rest and during Exercise in Patients with Arterial Hypertension

By

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Methyldopa (= α-methyl 3 4-dihydroxy DL-phenylalanine) has recently been introduced for treatment of arterial hypertension (Oates et al. 1960, Sjoerdsma 1960 Gillespie 1960 Gillespie and Sjoerdsma 1961) Its mode of action is not clearly established but it seems to be capable of inhibiting one step in the biosynthesis of norepinephrine In the course of our studies on the hemodynamics of arterial hypertension in man the influence of methyldopa on blood pressure and cardiac output at rest and during exercise in six patients with arterial hypertension was investigated.

Material

Six inpatients, five males and one female with established arterial hypertension were investigated. Their ages ranged from 4 to 61 years. All had essential hypertension, they had normal intravenous pyelograms, no signs of renal artery stenosis on renal aortograms, normal excretion of catecholamines and no signs of any other renal or adrenal disease. The heart size ECG-findings and pre-treatment blood pressure levels are listed in table 1. None had received hypotensive drugs for several weeks before the trial with methyldopa.

Submitted for publication July 19 1961

Methods

After at least two days of rest in the hospital the patients underwent the pre-treatment investigation, which was done in the morning in the fasting state. Under local anaesthesia and by the percutaneous route one catheter was placed in the right atrium and another in the brachial artery. Intravascular pressure recordings, determination of cardiac output using dye dilution technique with bromsulphalein as indicator according to Werkö (1956) and Mellette et al. (1958) and measurement of oxygen consumption by the sampling of expired air in Douglas bag were made with the subject sitting comfortably in chair. Mean blood pressure was calculated from pressure curves recorded with electrical damping and heart rate from simultaneously recorded ECG.

The same procedure was repeated during exercise in the sitting position upon bicycle ergometer according to Holmgren and Mattsson (1954. The intravascular blood pressure was as rule recorded after 1 3, 5, 7 and 8 min. of exercise. Sampling of expired air was started after 5 min. and cardiac output was determined after 10 min of exercise.

The administration of methyldopa was started the following day. No other hypotensive or sedative agents were given. The dose of

Will be introduced as Aldomet by Merck, Sharp and Dohme

dominal aorta. It should also be remembered that the renal arteries occupy a most unique hemodynamic position It has been maintained that their origin at right angles from the abdominal aorta, in contrast to all other branches is due to the necessity for the kidneys to utilize not only the downward current in the abdominal aorta but also the rebound phenomenon from the bifurcation both meet at the level of the renal arteries, where accordingly particular turbulence may be presumed In this connection attention may be called to the peculiar observation of Danaraj and collaborators (8) of a "Takayashu arteritis in two children confined not to the usual localization in the aortic arch but to the abdominal aorta at the origin of the renal arteries.

Summary and conclusions

1 A review is given of the various causes of symptomatic arterial hypertension

2 Two cases are described who had in common a high blood pressure a reduced size of the aorta, multiple aneurysms and interference with the renal blood supply In one of these cases there was also a coarctation of the infantile type.

3 The aortic hypoplasia and its importance for the development of arterial hypertension is discussed Attention is called to the wide caliber of the inferior vena cava

4 The abdominal coarctation and its development is discussed Attention is called to the peculiar hemodynamic conditions at the origin of the renal arteries.

5 In one of our cases the aldosteron output was determined It was found to be considerably increased in connection with the strangulation of the renal arterial blood supply It became normalized after removal of the responsible kidney This observation underlines the involvement of a suprarenal factor in the establishment of hypertension of renal origin [1]

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[1] We are indebted to our colleague Holfelt for the analysis of the aldosteron.

BP on admission and the day before 1st study		Dose per day of methyldopa	BP the day before 2nd study		Side effects
Lying	Standing		Lying	Standing	
190 115→155/90	160/105→140/85	1.5 g for 12 days	135/70	120/80	N
215/145→190/115	220/130→175/105	1.0 g for 5 days, 2.0 g for 8 days	175/115	165/110	No
140 100→140/85	145/95 →135/80	1.5 g for 12 days	120 70	120/70	Slight vertigo
170 125→165/105	170/130→165 105	2.0 g for 16 days	120 90	95 70	Orthostatism
205 140→180/120	205 140→180/120	1.5 g for 9 days	150 95	135 80	No
175/110→150 95	160/105→155 100	0.75 g for 2 days, 1.0 g for 7 days	150 80	135 80	Slight sedation

an average of 35 mm Hg At rest the systolic pressure was lower during methyldopa treatment in four subjects and slightly higher in two, while on exercise t was lower in every patient. Although one patient had the same diastolic pressure at rest before and during treatment, all subjects showed reduced diastolic pressures during exercise. In every patient the reduction of the systolic pressure was greater than of the diastolic, the average figures being 22 and 13 mm Hg at rest, 47 nd .3 mm Hg during exercise.

The right atrial pressure did not show any consistent changes.

Already at rest two patients had a somewhat slower heart rate during methyldopa treatment. This became more marked during exercise where the heart rat re corded at the stage of cardiac output determination was slower in five of six patients. In subject G H. the drop in heart rate was considerable from 180 to 146 beats/min. at exactly the same exercise load as demonstrated by the same degree of rise in oxygen consumption (1,390 and 1,389 ml O_2/min)

The effect upon blood pressure and heart rat is also shown in graphic form in fig 1 where the marked difference between pre treatment and treatment values in most patients is clearly seen. Here the values during exercise represent for each patient the a erage of the five recordings (except for subject E. L., where only four recordings were obtained) taken before determination of cardiac output in order to show that the new and lower blood pressure level is present during the whole exercise test.

Table I Data upon the six hypertensive patients studied

Subject	Sex	Age	K. W.[1]	Highest recorded BP mm/Hg	ECG	Heart size in ml/m² body area	Serum creatinine in mg %
E.L.	M	51	I	260/—	Normal	480	1.0
H.A.	M	60	I	250/170	Atr fibrill., left ventr hypertrophy and strain	780	1.3
V O	M	52	0	180 105	Normal	590	1.3
S M.	M	50	I	200/120	Left ventr hypertrophy and strain	400	1.2
G H.	M	24	0	205/140	Left ventr hypertrophy and strain	440	1.3
A A.	F	61	II	270 140	Left ventr hypertrophy and strain	430	0.9

[1] Group according to Keith Wagener

methyldopa varied between 0 75 to 2 0 g daily for 9—16 days divided in 3—4 oral doses per day (see table I) The blood pressure was taken twice daily in the lying and standing position by the auscultatory method

Under the same conditions and with the same technique as described above the hemodynamic study was repeated after 9—16 days, when the patients were under influence of methyldopa. The setting of the ergometer load was the same for every patient during both studies.

Results

In all patients but one (subject H A.) the blood pressure was lower on the day before the 2nd study than before the 1st (see table I) In three patients side effects appeared which were slight in two but the third showed a rather pronounced orthostatic reaction (subject S M.)

The results from the hemodynamic investigations with blood pressure, heart rate, cardiac output and oxygen consumption at rest and during exercise before and during treatment with methyldopa are listed in table II where the values given for brachial artery pressure during exercise are from the recording immediately before determination of cardiac output, i. e. as a rule after 8 min. of exercise.

The mean blood pressure at rest was always lower at the 2nd study but the difference was relatively small ranging from 5 to 37 mm Hg with an average of 16 mm Hg The difference between pre treatment and treatment values, however was considerably greater during exercise, where the reduction in mean blood pressure was 17—59 mm Hg with

BP on admission and the day before 1st study		Dose per day of methyldopa	BP the day before 2nd study		Side effects
Lying	Standing		Lying	Standing	
190/115→155/90	160/105→140/85	1.5 g for 12 days	135/70	120/80	No
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The effect upon blood pressure and heart rate is also shown in graphic form in fig 1 where the marked difference between pre-treatment and treatment values in most patients is clearly seen. Here the values during exercise represent for each patient the average f the five recordings (except for subject E. L. where only four recordings were obtained) taken before determination of cardiac output in order to show that th new and lower blood pressure level is present during the whole exercise test.

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A. A.	F	61	II	2 0/140	Left ventr hypertrophy and strain	450	0.9

Group according to Keith Wagener

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Under the same conditions and with the same technique as described above the hemodynamic study was repeated after 9—16 days, when the patients were under influence of methyldopa. The setting of the ergometer load was the same for every patient during both studies.

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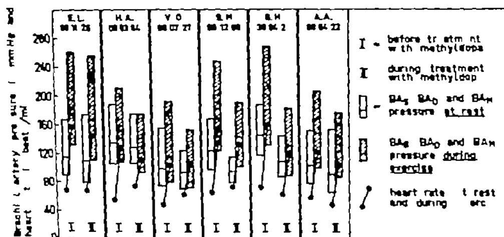


Fig. 1. The effect of methyldopa upon brachial artery pressure and heart rate at rest and during exercise, the values during exercise being the average of five recordings (except in subject E. L., where only four recordings were taken).

Fig. 2. The result of the hemodynamic studies at rest and during exercise in subject G. H., where in addition the exercise was repeated during constant infusion of minute amounts of angiotensin.

BA = brachial artery pressure in mm Hg
RA = right atrial pressure in mm Hg
HR = heart rate in beats/min.
O = oxygen consumption in ml O_2/min
AV = arteriovenous oxygen difference in ml O_2/l of blood.
CO = cardiac output in l/min.
SV = stroke volume in ml

Table II Results from the hemodynamic studies at rest and during exercise **before** *(= 1st study) and* **during** *(= 2nd study) treatment with methyldopa*

Subject		At rest								During exercise							
		HR	RA	BA_S	BA_D	BA_M	O_2-cons.	CO	SV	HR	RA	BA_S	BA_D	BA_M	O_2-cons.	CO	SV
E. L.	1st study	68	− 5	167	90	115	256	5.4	80	169	− 5	285	148	220	—	15.8	93
	2nd study	67	− 3	175	80	110	242	6.2	93	162	± 0	275	113	172	—	14.9	92
H. A.	1st study	55	− 4	188	106	135	310	3.8	69	136	+ 1	212	109	141	1 082	7.7	56
	2nd study	74	− 6	175	106	129	308	3.9	53	126	− 2	175	91	121	1 069	8.3	65
V. O.	1st study	48	− 1	155	73	99	286	5.4	113	126	− 8	207	78	116	1 689	12.0	97
	2nd study	62	—	124	71	91	275	6.6	106	118	—	158	71	99	1 618	13.5	114
S. M.	1st study	65	± 0	169	98	124	305	5.5	85	145	+ 5	262	125	185	1,869	14.6	101
	2nd study	73	± 0	115	80	95	260	4.4	60	153	+ 3	210	107	155	1,864	12.0	78
G. H.	1st study	73	− 2	188	118	146	347	7.2	98	180	− 1	280	129	179	1 737	13.2	73
	2nd study	62	− 2	145	88	109	257	5.4	87	146	− 1	193	90	120	1 646	12.8	88
A. A.	1st study	59	− 5	152	79	104	191	3.6	60	133	± 0	215	103	156	759	9.0	68
	2nd study	48	+ 1	153	66	91	175	4.0	82	110	+11(?)	171	81	122	897	9.1	83

HR = heart rate in beats/min. RA = right atrial pressure in mm Hg. $BA_{S, D \text{ and } M}$ = systolic, diastolic and mean brachial artery pressure in mm Hg. O_2-cons. = oxygen consumption in ml/min. CO = cardiac output in l/min. SV = stroke volume in ml.

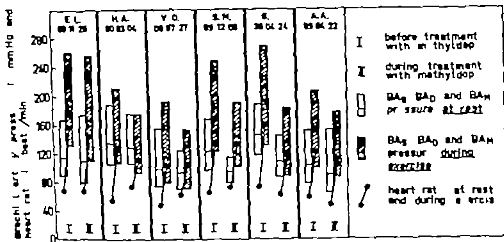


Fig. 1. The effect of methyldopa upon brachial artery pressure and heart rat rest and during exercise, the values during exercise being the average of fiv recordings (except in subject E. L., here only four recordings were taken)

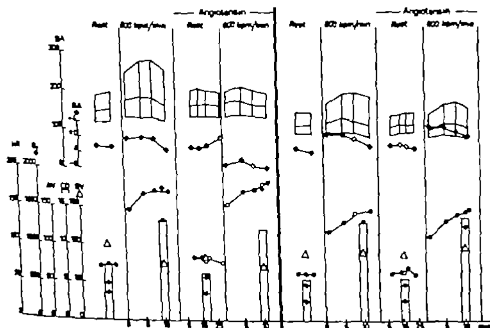


Fig. 2. The result of the hemodynamic studies at rest and during exercise in subject G. H., where in addition the exercise was repeated during constant infusion of minute amounts of angiotensin.

BA = brachial artery pressure in mm Hg
RA = right atrial pressure in mm Hg
HR = heart rate in beats/min
O = oxygen consumption in ml O_2/min.
AV = arteriovenous oxygen difference in ml O_2/ of blood
CO = cardiac output in l/min.
SV = stroke olume in ml

The cardiac output did not alter according to any special pattern during methyldopa and the average difference was negligible. However in two patients (S M and G H.) there was a reduction in cardiac output both at rest and during exercise amounting to 1 1 and 1 8 respectively 2 6 and 0 4 l/min It must be pointed out that both of them had considerably lower oxygen consumption at rest on the 2nd study and this could be a possible explanation of the diminished output Four patients had a moderate increase of stroke volume during exercise on methyldopa as compared with the 1st study while at rest only two of them showed such an increase

Discussion

The results show that methyldopa has a definite hypotensive effect which may sometimes be very pronounced as in subject G H where the blood pressure decreased from 188/118 to 145/88 mm Hg at rest and from 280/129 to 193/90 mm Hg during exercise. This means almost normalization of the blood pressure and its reaction upon exercise with methyldopa in a dose of 1 5 g per day In this patient the acute hemodynamic effect of angiotensin given in minute amounts as a constant infusion was also studied and the exercise test repeated on the same ergometer load after about one hour of rest. As shown in the right part of fig 2 the rise in brachial artery pressure was almost the same, confirming that a consistent blood pressure reduction was reached

The present study was conducted as the exercise blood pressure may be a better indicator of the usefulness of a particular medication. Sometimes the effect of methyldopa seemed to be rather slight as judged from the values at rest (subjects H. A. and A. A.) During exercise, however the response of blood pressure was normalized showing that the drug is an effective hypotensive agent.

A significant reduction in heart rate at rest in five patients given methyldopa intravenously has been reported by Wilson et al (1961) Only in two of our patients was a decrease in heart rate at rest observed following methyldopa administered orally for several days. On exercise, however the heart rate was slower in five of six patients performing the same amount of work, and in one subject this reduction was substantial (from 180 to 146 beats/min.) Depletion of cardiac catecholamines by blockade of the synthesis and by a possible methyldopa action on the storage mechanism (Sjoerdsma 1960) may be responsible for the decrease in heart rate. As exercise is accompanied by an increased demand of catecholamine action on the heart the effect of a certain dose of methyldopa may be more pronounced during exercise.

Cardiac action of methyldopa could be expected to decrease cardiac performance in terms of diminishing cardiac output in analogy with the effect of ganglionic blockade (Beck 1958) Wilson et al (1961) found in acute studies that the decrease in blood pressure produced by methyldopa was caused by a decrease in cardiac output. Our results obtained after more long standing treatment show no such decrease in most of the hypertensive patients studied. Stroke volume actually increased simultaneously with reduction in heart rate in four patients resulting in a virtually unchanged cardiac output in most instances on exercise as compared with the corresponding pre-treatment values. This suggests, that the decrease in blood pressure is mainly produced by methyldopa action on peripheral resistance. On plot

ting flow against pressure as done in fig 3 the slopes then represent the changes in resistance and it is seen that in all cases but one the slopes from the 2nd study during treatment with methyldopa clearly tend to have a less steep rise indicating a lowered total peripheral resistance.

In studies on the effect of chlorothiazide (Varnauskas et al. 1961) where the same methods and procedure of investigation as in this study were used, a decrease in cardiac output and stroke volume as well as lowering of blood pressure were observed in all eight patients both at rest and on exercise during the administration of chlorothiazide or chlorthalidone. The effect of methyldopa differs from that of chlorothiazide or chlorthalidone in the absence of decrease of right atrial pressure or cardiac output but agrees in the decrease of peripheral resistance and blood pressure. In view of these facts the results of the present study seem to be significant despite the relatively small number of patients (6) studied.

Side effects were seen in three patients and consisted of slight vertigo orthostatism and slight sedation. No pronounced sedation was observed, nor any mental depression.

The introduction of methyldopa makes a new interesting approach in the treatment of hypertension, because it is an attempt to attack directly in a biological way one of the probable etiological factors. Before any assessment can be made on the place of this drug in the treatment of arterial hypertension more longterm treatment results must be reported. Possible side reactions may be found which may preclude the use of this otherwise promising substance.

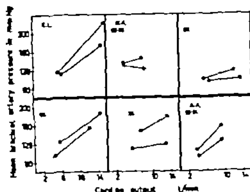


Fig 3. Cardiac output at rest and during exercise plotted against mean brachial artery pressure the slopes representing changes in total peripheral resistance.

● = before treatment with methyldopa
○ = after treatment with methyldopa.

Summary

1 The hemodynamic effects of methyl dopa, which is said to be an inhibitor of amine biosynthesis, have been studied in six patients with essential hypertension.

2. The hemodynamic study included determination of brachial artery and right atrial pressures, cardiac output, heart rate and oxygen consumption at rest and during exercise in the sitting position and were performed before and during oral administration of 0 75—2.0 g of methyldopa per day

3 At the 2nd study mean blood pressure at rest was lower in all patients and the reduction was more pronounced during exercise, the average reduction being 16 and 35 mm Hg respectively The systolic blood pressure was always reduced more than the diastolic. The right atrial pressure did not show any consistent changes.

4 At rest only two patients had a slower heart rate during methyldopa treatment, but on exercise five of six patients showed a slower heart rate when doing the same amount of work as before methyldopa.

5 The changes in cardiac output did not show any special pattern, and the average difference was negligible. Stroke volume increased in four of six patients on exercise simultaneously with the slower heart rate.

6 It is proposed, that the decrease in blood pressure is mainly produced by methyldopa action on peripheral resistance.

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The Proactivator of the Fibrinolytic System in Human Plasma

The Quantitative Determination and its Clinical Application

By

Sverre Blix

The existence of a proactivator (Müllertz & Lassen 1953) as an inactive human plasma factor which interacts with streptokinase to form an activator of plasminogen has now been generally accepted. However it has not been proved whether this factor constitutes a part of the human plasminogen molecule or not. The interaction between proactivator and streptokinase is stoichometric, while the activation of plasminogen is enzymatic (Müllertz 1955, Troll & Sherry 1955). The streptokinase-proactivator interaction has been studied in detail by Lassen (1959 a, b) and Greig & Cornelius (1960) have separated a fraction with proactivator properties from human plasma. Human plasma contains relatively small amounts of plasminogen and large amounts of proactivator while bovine plasma contains large amounts of plasminogen, but no or insignificant amounts of proactivator (Müllertz 1956).

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The purpose with the present study has been to determine the proactivator concentration in plasma under various conditions. For this purpose it was necessary to use a reliable assay system. Christensen (1949) used bovine fibrinogen, which usually is contaminated with plasminogen (Astrup & Permin 1947) as substrate in his systems for determination of plasminogen and streptokinase. Later Blombäck et al. (1955), Nilsson et al. (1957) and Lassen (1958) have used bovine fibrinogen as substrate in their plasminogen-proactivator or proactivator methods. The present method with bovine plasma as substrate is based upon the same principles as these methods. The method is not influenced by the test plasma concentration of plasminogen, as such, but if proactivator is a part of the human plasminogen molecule the results obtained in this report will also reflect the plasminogen

4 At rest only two patients had a slower heart rate during methyldopa treatment but on exercise five of six patients showed a slower heart rate when doing the same amount of work as before methyldopa.

5 The changes in cardiac output did not show any special pattern, and the average difference was negligible. Stroke volume increased in four of six patients on exercise simultaneously with the slower heart rate.

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Fibrinogen determination. Fibrinogen was determined as fibrin after coagulation with thrombin by the method of Jacobsson (1955) with the modifications of Blombäck & Blombäck (1956) and Godal (1961). 0.1 ml 5 per cent epsilon-amino-caproic acid was added to 0.4 ml of plasma to avoid fibrinolysis of the clot.

Fibrinolytic investigations were performed on fibrin plates, as described by Astrup & Müllertz (1952) and Lassen (1952). The heated plates were kept for one hour at 85° C. In the present study these heated plates were slightly more sensitive to proteolytic enzymes than the standard plates.

Proconvertin-prothrombin (PP) was determined by the method of Owren & Aas (1951).

Standard deviation was calculated from this formula:

$$S.D. = \sqrt{\frac{\Sigma(\bar{X}-Y)^2}{n-1}}$$

and the coefficient of variation $\frac{S.D. \cdot 100}{\bar{Y}}\%$

Y: readings

$\bar{X}$: mean of readings,

n: number of readings.

Determination of proactivator in plasma

A. DESCRIPTION OF THE METHOD

1. *The test system*

The system:

0.1 ml citrated bovine plasma,

0.05 ml citrated human test plasma, diluted in proportion one part of plasma and nine (or nineteen) parts of buffer

0.05 ml streptokinase (8,000 units/ml)

0.1 ml thrombin (30 N.I.H. units/ml)

2. *The principle of the method*

Streptokinase added in excess (80,000 units per ml human plasma) of proactivator to the human test plasma rapidly forms the activator of plasminogen (Gerheim & Ferguson 1949). The human test plasma is the only source of proactivator in the system. Plasminogen is present in the bovine plasma, in the bovine thrombin (see later) and in insignificant amounts in the human test plasma, as this plasma contains

Table I. Four different bovine plasmas as substrate in the system. The same human test plasma was used in all experiments

Bovine plasma (No.)	Fibrinogen (mg %)	Clot lysis time (sec.)
1	635	175
2	755	177
3	488	155
4	371	204

small amounts of plasminogen and is further diluted twenty times in relation to the bovine plasma. The plasminogen is converted to plasmin by the activator and plasmin dissolves the fibrin rapidly. Fibrin is formed from the fibrinogen in the bovine plasma, and only in insignificant amounts from fibrinogen in the diluted human plasma (see later).

3. *Performance for the test*

All reagents were kept in ice water. The bovine plasma was incubated for 15 seconds at 37° C in glass test tubes (10 × 70 mm) and the other materials were added at 15 seconds intervals. The time from addition of thrombin to complete lysis of the clot was recorded. Soon after the clot had formed, small air bubbles could be observed in the clot, as described by Blombäck et al. (1955) and Lassen (1958). A few seconds before the clot became lysed all the air bubbles rose to the surface. When they reached the surface, the clot was completely lysed, and this point was easy to record. All the tests were performed in duplicate.

The same batches of bovine plasma, standard plasma, streptokinase and thrombin were used throughout the study.

B. THE SUBSTRATE

1. *The bovine plasma*

Bovine plasma contains large amounts of fibrinogen and plasminogen, but no or insignificant amounts of proactivator. The lysis times obtained with four different bovine plasmas tested with the same human plasma are given in table I. The variation in lysis times probably reflects the combined effect of plasminogen, fibrinogen and inhibitors in the bovine plasmas.

content. To facilitate an interpretation of the results in the present study a series of control experiments has been performed.

The clinical investigation comprises examination of normal subjects and of patients on anticoagulant treatment, with bleeding disorders, with high fibrinogen levels, with fibrinogenopenia, and with liver diseases The proactivator concentration was found to be low in patients with impaired liver function. Therefore the possibility of using proactivator determination as a liver function test has been proposed.

Materials

Anticoagulant 1) sodium citrate dihydrate 3 13 g per cent, 2) potassium oxalate monohydrate 2 5 per cent, 3) heparin (A. L. Oslo, Norway) 25 I U per ml saline.

Buffer A modified veronal buffer (pH 7.35 and ionic strength 0 154) was made by mixing sodium diethyl barbiturate 11 75 g sodium chloride 14 67 g 0 1 N HCl 430 ml and distilled water to 2 000 ml (Owren 1947)

Epsilon-amino-caproic acid (Light & Co. Ltd., Colnbrook, England) was made up as a 5 per cent solution in saline, glass filtered (G_4) and autoclaved at 120° C for 20 minutes.

Fibrinogen. Bovine fibrinogen for the fibrin plates was prepared by N Gröndahl, Spånga, Sweden. The fibrinogen had a clottability of 97 per cent, and was stored at —20° C in a 1.2 per cent solution in buffer

Streptokinase "Varidase (Lederle Laboratories, New York, U S. A.) containing 20 000 units of streptokinase and 5 000 units of streptodornase per vial was used. The material was dissolved and diluted to desired concentrations in buffer New solution was prepared every day In order to avoid loss of activity due to adsorption of streptokinase to glass (Larsen 1958) lusteroid tubes (Beckman Instruments Inc., Fullerton, California, U S A.) were used for streptokinase. Streptokinase was not adsorbed to these tubes, and adsorption to glass pipettes was of no practical importance for the present method.

Thrombin. "Topostasin" (Roche, Switzerland) containing 3 000 N I H. units per vial was disolved in buffer to 150 units per ml and stored in aliquots at —20° C. New solution was prepared every week.

Urokinase was prepared by the method of Astrup & Sterndorff (1952)

Methods

Collection of blood Nine parts of blood were mixed with one part of a 3 13 g per cent sodium citrate dihydrate solution. In this study citrate platelet poor plasma was used where nothing else is stated In one experiment heparin and potassium oxalate monohydrate (see Materials) were used as anticoagulant. The blood was chilled to 4 C and centrifuged as soon as possible at 4 C. Platelet poor plasma was obtained by centrifuging the blood for 30 minutes at 2,500 r p m. (1 400 G) Platelet-rich plasma was obtained by centrifuging the blood for 30 minutes at 600 r p m. (80 G) The plasma was at once pipetted off and tested, or stored in aliquots at —20° C, and thawed shortly before use.

Serum was obtained in two ways 1 "Saline serum" Nine parts of blood were mixed with one part of saline and allowed to clot during 15 minutes at room temperature. The blood was then centrifuged as for platelet-poor plasma, and the serum pipetted off. 2 "Citrated serum To obtain a serum sample with the same concentration of citrate as in plasma, undiluted whole blood was allowed to clot during 10 minutes at room temperature. The blood was the centrifuged as for platelet-poor plasma, and the serum pipetted off Finally citrate was added to the serum, in accordance with the haematocrit value.

Electrophoretic studies were performed in the Central Laboratories of the University Hospital and Oslo City Hospitals in an Elfor Apparatus (Hobein & Bender Deutschland) with a diemal buffer (pH 8.6 and ionic strength 0 1) The papers were stained with amido black.

Euglobulin fractionation was performed as previously described (Blix 1961) For preparation of a plasminogen-rich euglobulin fraction from bovin serum, the serum was precipitated at pH 5.2 in dilution 1 part of serum and 19 parts of acetic acid.

Fibrinogen determination. Fibrinogen was determined as fibrin after coagulation with thrombin by the method of Jacobsson (1955) with the modifications of Blombäck & Blombäck (1956) and Godal (1961). 0.1 ml 5 per cent epsilon-amino-caproic acid was added to 0.4 ml of plasma to avoid fibrinolysis of the clot.

Fibrinolytic investigations were performed on fibrin plates, as described by Astrup & Mullertz (1952) and Lassen (1952). The heated plates were kept for one hour at 85 C. In the present study these heated plates were slightly more sensitive to proteolytic enzymes than the standard plates.

Proconvertin-prothrombin (PP) was determined by the method of Owren & Aas (1951).

Standard deviation was calculated from this formula

$$S.D. = \sqrt{\frac{\Sigma(\bar{X}-X)}{n-1}}$$

and the coefficient of variation $\frac{S.D. \cdot 100}{\bar{X}}$ %

X readings
$\bar{X}$ mean of readings.
n number of readings.

Determination of proactivator in plasma

A. DESCRIPTION OF THE METHOD

1 *The test system*

The system
0.1 ml citrated bovine plasma,
0.05 ml citrated human test plasma, diluted to proportion one part of plasma and nine (or nineteen) parts of buffer
0.05 ml streptokinase (8,000 units/ml)
0.1 ml thrombin (30 N. I. H. units/ml)

2 *The principle of the method*

Streptokinase added in excess (80,000 units per ml human plasma) of proactivator to the human test plasma rapidly forms the activator of plasminogen (Gerheim & Ferguson 1949). The human test plasma is the only source of proactivator in the system. Plasminogen is present in the bovine plasma, in the bovine thrombin (see later) and in insignificant amounts in the human test plasma, as this plasma contains small amounts of plasminogen and is further diluted twenty times in relation to the bovine plasma. The plasminogen is converted to plasmin by the activator and plasmin dissolves the fibrin rapidly. Fibrin is formed from the fibrinogen in the bovine plasma, and only in insignificant amounts from fibrinogen in the diluted human plasma (see later).

Table I. Four different bovine plasmas as substrate in the system. The same human test plasma was used in all experiments

Bovine plasma (No.)	Fibrinogen (mg %)	Clot lysis time (sec.)
1	635	175
2	755	177
3	488	155
4	571	204

3. *Performance for the test*

All reagents were kept in ice water. The bovine plasma was incubated for 15 seconds at 37 C in glass test tubes (10 × 70 mm) and the other materials were added at 15 seconds' intervals. The time from addition of thrombin to complete lysis of the clot was recorded. Soon after the clot had formed, small air bubbles could be observed in the clot, as described by Blombäck et al. (1955) and Lassen (1958). A few seconds before the clot became lysed all the air bubbles rose to the surface. When they reached the surface, the clot was completely lysed, and this point was easy to record. All the tests were performed in duplicate.

The same batches of bovine plasma, standard plasma, streptokinase and thrombin were used throughout the study.

B. THE SUBSTRATE

1 *The bovine plasma*

Bovine plasma contains large amounts of fibrinogen and plasminogen, but no or insignificant amounts of proactivator. The lysis times obtained with four different bovine plasmas tested with the same human plasma are given in table I. The variation in lysis times probably reflects the combined effect of plasminogen, fibrinogen and inhibitors in the bovine plasmas.

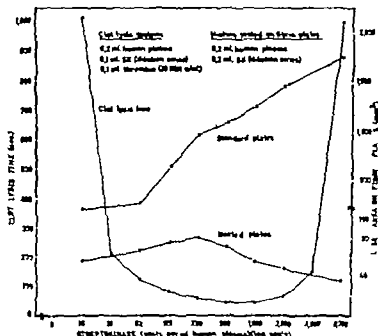


Fig 1 The effect of various concentrations of streptokinase in human plasma, as tested in a human plasma clot system (ordinate, left) and on *standard* and *heated fibrin plates* (ordinate, right)

2. *Streptokinase*

a) Streptokinase effect in human plasma.

Christensen (1949) reported an inhibitory effect of high concentrations of streptokinase in fibrinolytic systems. In a casein system Alkjaersig et al. (1959) showed that streptokinase added to human plasminogen formed increasing amounts of activator up to the highest concentration used However plasmin formed in the system reached a maximal activity with much smaller concentrations of streptokinase the activity decreased when higher concentrations were used.

The present investigation confirmed these results. The effect of streptokinase in a human plasma clot is demonstrated in fig 1 The same concentrations of streptokinase in human plasma were tested on standard and heated plates, and these results are also given in fig 1 It is apparent that increasing amounts of activator (standard plates) became formed up to the highest concentrations of streptokinase used Plasmin activity (heated plates) however showed a maximum of activity at 125—500 units of streptokinase per ml plasma.

To study the maximal activator activity attainable in plasma by *addition of streptokinase*, the plasma was diluted ten times with buffer Streptokinase of increasing concentrations was added and the mixtures tested on standard plates (fig 2) The maximal formation of activator was achieved at about 20 000 units of streptokinase per ml plasma. In the same figure the effect of streptokinase in the plasma from a patient with high streptokinase antibody level (Blix 1961 b) is demonstrated (see later)

b) Lack of streptokinase effect in bovine plasma.

No inhibitory effect of high concentrations of streptokinase was found in a bovine plasma clot system. The system and the results are given in fig 3 Due to the lack of proactivator in bovine plasma, streptokinase is *not* able to induce lysis of a bovine clot (Astrup & Permin 1948 Clifton & Cannamela 1951) Therefore addition of small amounts of human plasma for formation of activator was necessary

c) Streptokinase in the system.

In the proactivator system 4 000 units of streptokinase per ml bovine plasma was present, or 80,000 units of streptokinase per ml human plasma which was an excess compared with the proactivator concentration (fig 2)

3 *The thrombin*

The effect of various concentrations of *thrombin in the system is reported in table II* Increasing amounts of thrombin shortened the lysis time. However this bovine thrombin contained considerable amounts of plasminogen. This plasminogen was determined as plasmin

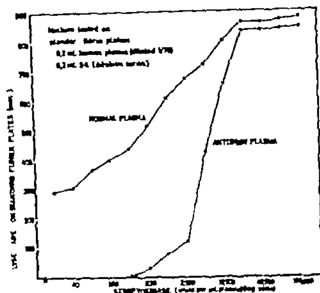


Fig. 2. Formation of activator after addition of streptokinase to human plasma as tested on standard fibrin plates.

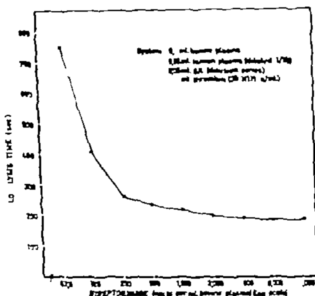


Fig. 3. Streptokinase activity in bovine plasma as tested in clot system after addition of small amounts of human plasma for supply of proactivator.

after activation with equal volumes of urokinase (Blix 1961 c); the mixtures were tested on heated plates. I fig 4 dilution series for plasminogen (the thrombin solution) tested as plasmin on heated plates is given.

This observation indicates that the composition of the thrombin preparation is of great importance for the lysis time in this system. In one batch of thrombin the plasminogen concentration was so high that the lysis times became too short. Such preparation may still be used, but the human plasma should be tested in higher dilution than usual, to give lysis times between 160 and 240 seconds.

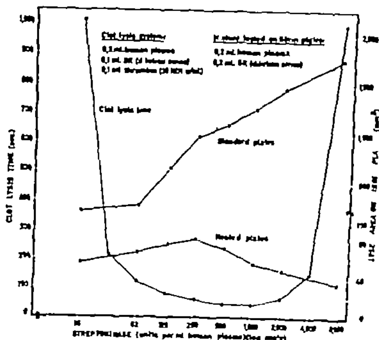


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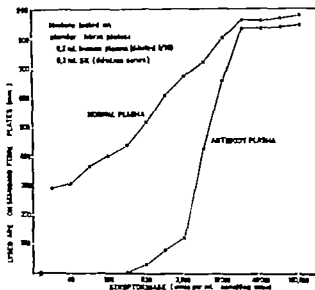


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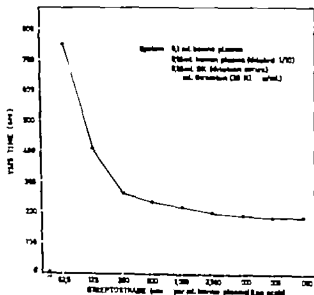


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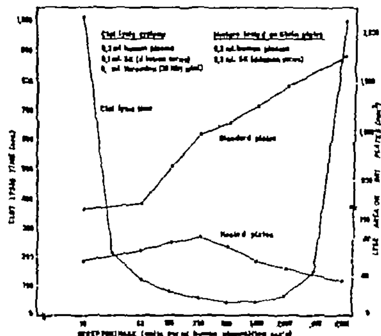


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and show that plasmas with proactivator content above 150 per cent should always be tested at the 50 per cent level.

The most convenient lysis time at the 100 per cent level was between 160 and 240 seconds. This can be obtained by using suitable bovine plasma, by changing the thrombin concentration (see under The substrat) or if necessary by using higher dilution of the test plasma.

In table IV the lysis times of 12 parallel tests at 100, 50 and 25 per cent levels are reported. The standard deviation and the coefficient of variation at the 100 per cent level were 4.2 seconds and 2.2 per cent respectively.

2. *Plasminogen*

The human plasma (at the 100 per cent level) was diluted twenty times compared with the bovine plasma. It was improbable, therefore, that the plasminogen, the fibrinogen or inhibitors in the human plasma were of importance for the results.

The euglobulin fraction of bovine serum contains large amounts of plasminogen. This plasminogen was determined as plasmin after activation with an equal volume of urokinase; the mixture was tested on heated fibrin plates. In fig. 5 human plasma, human euglobulin fraction, bovine serum euglobulin fraction and thrombin have been compared with respect to plasminogen content. It is apparent that the bovine serum euglobulin fraction contained more plasminogen than human plasma.

Euglobulin fraction from bovine serum was added to the diluted human plasma in order to find out whether this change in plasminogen concentration was of any importance.

Table III. Plasmas from pregnant women with high proactivator levels were tested at the usual 100 per cent level and also at the 50 per cent level

Patient (No.)	Proactivator in plasma (per cent)	
	100 % level (plasma dil. 1:9)	50 % level (plasma dil. 1:19)
1	124	128
2	128	137
3	129	124
4	137	133
5	137	135
6	139	147
7	142	143
8	147	145
9	157	177
10	158	179

0.2 ml citrated human plasma,
0.2 ml euglobulin solution from bovine serum,
1.6 ml buffer.
As control served
0.2 ml citrated human plasma,
1.8 ml buffer.

The lysis time in the proactivator system with the first mixture as test plasma was 201 seconds, and with the second mixture 204 seconds, indicating that plasminogen in the test plasma was of little importance.

3. *Fibrinogen*

Serum contained about 65 per cent of the proactivator content in plasma (see later). In fig. 6 the dilution curves for citrated plasma and citrated serum (see Methods) as test materials have been compared. The slope of the curves

Table IV. The results of twelve parallel tests with the same human test plasma in various dilutions

	Clot lysis time (sec.) (Twelve parallel tests)	Mean (sec.)	S. D. (sec.)	C. o. V (%)
100 level (human plasma dil. 1:9)	189—190—183—187—194—193 191—194—194—195—193—193	192	4.2	2.2
50 level (human plasma dil. 1:19)	256—252—254—253—258—252 244—240—258—244—251—252	251	5.7	2.3
25 level (human plasma dil. 1:39)	390—382—388—390—373—387 378—387—390—378—392—384	385	7.2	1.9

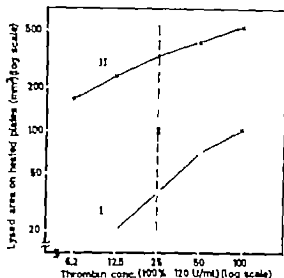


Fig 4. The curves show dilution series for the thrombin solution which contained plasminogen, after mixing with equal volumes of buffer (I) and urokinase (II) as tested on heated fibrin plates i. e., in curve II total plasminogen was determined as plasmin after activation with urokinase. The broken line is drawn through the point which corresponds to the concentration of the thrombin solution used in the proactivator test system. For comparison plasminogen in the euglobulin fraction of bovine serum (■) human plasma (●) and the euglobulin fraction of human plasma (○) tested in the same way are given.

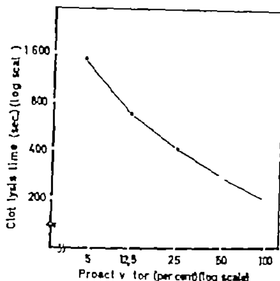


Fig 5 A standard curve for calculation of per cent proactivator in human plasma. The abscissa gives proactivator in per cent of normal, obtained by dilution of normal human plasma 100 per cent is plasma dilution 1 9 (one part of plasma and 9 parts of buffer)

C. THE HUMAN TEST PLASMA

1 *The standard curve*

Fig 5 gives a dilution curve of normal human plasma (the 100 per cent value was the lysis time of plasma diluted in proportion 1 part of plasma and 9 parts of buffer later referred to as plasma diluted 1 9) In the present study proactivator concentrations of all plasmas were determined on a standard curve the same standard plasma was used throughout the whole investigation (concerning normal range see application of the method)

Plasmas with high proactivator concentrations were usually tested at the 50 per cent level, as the standard curve stopped at 100 per cent. However experiments showed that for practical purposes the curve could be prolonged beyond this value, and higher proactivator concentrations determined by extrapolation with reliable results. Ten plasmas with high proactivator concentrations (pregnant women) were tested at the usual 100 per cent level (dilution 1 9) and the lysis times were translated into per cent proactivator from the prolonged standard curve. The same plasmas were also tested at the usual 50 per cent level (one part of plasma and nineteen parts of buffer later referred to as plasma diluted 1 19) the lysis times were interpolated and calculated in per cent proactivator on the standard curve, and the per cent value multiplied with 2 The results are given in table III

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5	1 9	243
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and show that plasma with proactivator content above 150 per cent should always be tested at the 50 per cent level.

The most convenient lysis time at the 100 per cent level was between 160 and 240 seconds. This can be obtained by using suitable bovine plasma, by changing the thrombin concentration (see under The substrate) or if necessary by using higher dilution of the test plasma.

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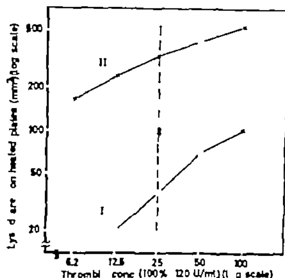


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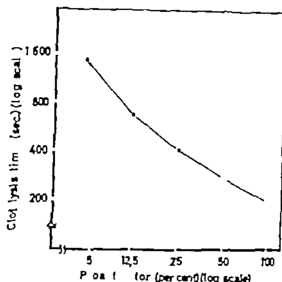


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2. Plasminogen

The human plasma (at the 100 per cent level) was diluted twenty times compared with the bovine plasma. It was improbable therefore that the plasminogen, the fibrinogen or inhibitors in the human plasma were of importance for the results.

The euglobulin fraction of bovine serum contains large amounts of plasminogen. This plasminogen was determined as plasmin after activation with an equal volume of urokinase: the mixture was tested on heated fibrin plates. In fig. 5 human plasma, human euglobulin fraction, bovine serum euglobulin fraction and thrombin have been compared with respect to plasminogen content. It is apparent that the bovine serum euglobulin fraction contained more plasminogen than human plasma.

Euglobulin fraction from bovine serum was added to the diluted human plasma in order to find out whether this change in plasminogen concentration was of any importance.

Table III. Plasmas from pregnant women with high proactivator levels were tested at the usual 100 per cent level and also at the 50 per cent level

Patient (No.)	Proactivator in plasma (per cent)	
	100 level (plasma dil. 1:9)	50 level (plasma dil. 1:19)
1	1?4	128
2	128	137
3	129	1?4
4	137	135
5	137	135
6	139	147
7	142	143
8	147	145
9	157	177
10	158	179

0.2 ml citrated human plasma
0.2 ml euglobulin solution from bovine serum,
1.6 ml buffer
As control served
0.2 ml citrated human plasma,
1.8 ml buffer

The lysis time in the proactivator system with the first mixture as test plasma was 201 seconds, and with the second mixture 204 seconds, indicating that plasminogen in the test plasma was of little importance.

3. Fibrinogen

Serum contained about 65 per cent of the proactivator content in plasma (see later). In fig. 6 the dilution curves for citrated plasma and citrated serum (see Methods) as test materials have been compared. The slope of the curves

Table IV. The results of twelve parallel tests with the same human test plasma in various dilutions

	Clot lysis time (sec.) (Twelve parallel tests)	Mean (sec.)	S. D. (sec.)	C. a. V. (%)
100 % level (human plasma dil. 1:9)	189–190–183–187–191–193 191–194–194–195–193–193	192	4.2	2.2
50 % level (human plasma dil. 1:19)	256–252–254–253–258–252 244–240–258–244–251–252	251	5.7	2.3
25 level (human plasma dil. 1:39)	390–382–388–398–373–387 376–387–390–378–392–381	385	7.2	1.9

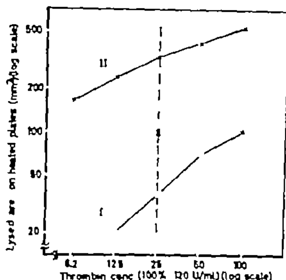


Fig 4 The curves show dilution series for the thrombin solution wh ch contained plasminogen, after mixing with equal volumes of buffer (I) and urokinase (II) as tested on heated fibrin plates i e., in curve II total plasminogen was determined as plasmin after activation with urokinase. The broken line is drawn through the point which corresponds t the concentration of the thrombin solution used in the proactivator test system For comparison plasminogen in the euglobulin fraction of bovin serum (■) human plasma (●) and the euglobulin fraction of human plasma (○) tested in the same way are given

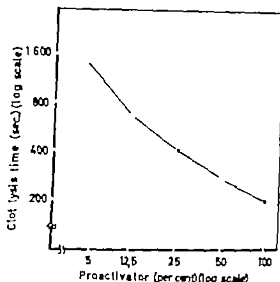


Fig. 5 A standard curve for calculation of per cent proactivator in human plasma. The abscissa gives proactivator in per cent of normal, obtained by dilution of normal human plasma 100 per cent is plasma dilution 1 9 (one part of plasma and 9 parts of buffer)

C. THE HUMAN TEST PLASMA

1 *The standard curve*

Fig 5 gives a dilution curve of normal human plasma (the 100 per cent value was the lysis time of plasma diluted in proportion 1 part of plasma and 9 parts of buffer later referred to as plasma diluted 1 9) In the present study proactivator concentrations of all plasmas were determined on a standard curve the same standard plasma was used throughout the whole investigation (concerning normal range see application of the method)

Plasmas with high proactivator concentrations were usually tested at the 50 per cent level as the standard curve stopped at 100 per cent However experiments showed that for practical purposes the curve could be prolonged beyond this value, and higher proactivator concentrations determined by extrapolation with reliable results. Ten plasmas with high proactivator concentrations (pregnant women) were tested at the usual 100 per cent level (dilution 1 9) and the lysis times were translated into per cent proactivator from the prolonged standard curve. The same plasmas were also tested at the usual 50 per cent level (one part of plasma and nineteen parts of buffer later referred to as plasma diluted 1 19) the lysis times were interpolated and calculated in per cent proactivator on the standard curve, and the per cent value multiplied with 2 The results are given in table III

Table II Effect of various concentrations of the thrombin solution in the test system

Thrombin conc. (N I H u/ml)	Human plasma dilution	Clot lysis time (sec)
5	1 9	243
	1 19	336
20	1 9	210
	1 19	264
50	1 9	162
	1 19	207

Table VI. Four normal plasmas (containing 116, 91, 116 and 96 per cent proactivator) and their corresponding sera were tested for proactivator. For convenience the proactivator in each plasma is called 100 per cent in the table and the proactivator in serum calculated in per cent of that

Subject (No.)	Proactivator in plasma (per cent)	Proactivator in serum (per cent)
1	100	63.5
2	100	63.7
3	100	64.7
4	100	68.4

Table VII. The proactivator content in the various euglobulin fractions in per cent of proactivator in plasma (see text)

	The various fractions (pH)			
	7.6—6.8	6.8—6.2	6.2—5.7	5.7—5.1
Proactivator precipitated	<10	29	11"	<10"

7. *Platelet-poor and platelet-rich plasma*

I normal platelet-rich plasma the proactivator levels were 0—10 per cent lower than in platelet poor plasma. The same was found if the platelet-rich plasma had been frozen.

8. *Stability of proactivator in plasma*

Plasma was stored at —20° C, 4° C, 20° C and 37° C for 4 hours. At various intervals the proactivator concentration was determined. No change in proactivator level during incubation was recorded, indicating high stability of proactivator in accordance with Mullertz (1954). At —20° C plasma could be stored for months without detoriation of the proactivator.

B. SERUM OR EUGLOBULIN FRACTION OF PLASMA AS TEST MATERIAL

1. *Proactivator concentration in serum*

Plasma and serum (saline serum) were prepared as described in Methods. The proactivator was determined in plasma and in the corresponding serum. Table VI shows that the proactivator concentration in serum was about 65 per cent of that in plasma (see also fig. 6).

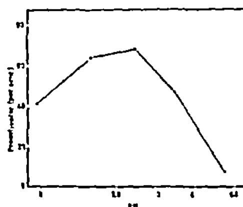


Fig. 7. Yield of proactivator in the euglobulin fractions from human plasma (proactivator 100 per cent) precipitated with acetic acid in dilution 1:10 at various pH values (abscissa).

2. *Proactivator concentration in the euglobulin fraction of plasma*

a) Plasma was diluted with various concentrations of acetic acid in proportion 1:19 and the euglobulin solution prepared as previously described (Blix 1961). The proactivator concentration in the various euglobulin solutions was determined. Fig. 7 shows that up to 68 per cent of proactivator in plasma could be precipitated.

b) Fractionated euglobulin precipitation as previously described (Blix 1961) was performed and the proactivator concentration in the various fractions determined. The results are given in table VII and show that the greatest amount of proactivator was in the fraction precipitated at pH 6.8—6.2.

Application of the method

A. PROACTIVATOR DETERMINATION IN PLASMA FROM NORMAL SUBJECTS

1. *Adults*

In the present study one batch of plasma from normal subject has been used for determination of standard curves. This plasma was stored in aliquots at —20° C. Each morning a new standard curve was made, though the curves varied little from day to day.

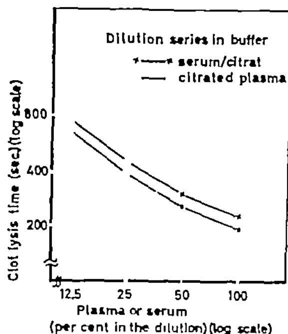


Fig 6 Dilution curves of plasma and serum (see text) in the proactivator system.

was the same, which probably should not be expected if fibrinogen in the diluted human plasma was of importance for the results. The results obtained with citrated serum or saline serum (see Methods) were practically identical indicating that the citrate in the human plasma or serum did not influence the results.

Addition of fibrinogen to serum would not have given reliable information, as we could not obtain bovine fibrinogen without plasminogen, or human fibrinogen without plasminogen and proactivator contamination However also theoretically it would seem unlikely that fibrinogen in the human plasma, which was diluted twenty times compared with the bovine plasma, should be of practical importance for the results.

4 *Inhibitors*

Proactivator was determined in plasma from two patients with high levels of inhibitors, in order to find out the influence of inhibitors on the test system.

a) Streptokinase antibodies.

Proactivator determination was performed in plasma from a patient with a high concentration of streptokinase antibodies after streptokinase treatment (Blix 1961 c) He had a proactivator concentration of 112 per cent of normal (see later) Although his true proactivator concentration was not known, this normal result suggests that streptokinase antibodies are of little importance for the test system. When the same plasma was tested in Lassen's proactivator system (Lassen 1958) the proactivator concentration was only 30 per cent of normal, probably due to the much lower streptokinase concentration in that system.

Table V Proactivator in human plasma, tested in various dilutions, before and after injection of 2 g epsilon-amino-caproic acid

	Proactivator (per cent) tested in plasma of various dilutions			
	1 9	1 19	1 39	1:79
Before E.-a.-c. a.	138	140	136	120
After E.-a.-c. a.	48	66	88	120

b) Epsilon-amino-caproic acid

Forty ml of a 5 per cent solution (2 g) was injected i.v during 10 minutes to a patient with cancer of the prostate in a phase with high fibrinogen level (487 m per cent) and a high proactivator concentration (138 per cent of normal) This amount of epsilon-amino-caproic acid strongly inhibited the fibrinolytic activity in vivo (Blix 1961 a) Blood was collected just before and five minutes after the injection. The results of the proactivator determinations showed that this concentration of inhibitor in the test plasma inhibited the test system, as the second blood sample showed only 48 per cent proactivator However when dilution series were tested identical results were obtained in both plasmas in the dilution 1 79 (i. e. at the 12.5 per cent level) The results of these proactivator determinations are given in table V In practice, such high levels of inhibitors will probably never occur

5 *Anticoagulant*

The same proactivator level in plasma was recorded whether blood was collected with citrate, oxalate or heparin as anticoagulant.

6 *Silicone or glass ware*

The same proactivator level in plasma was recorded whether siliconized equipment was used or not.

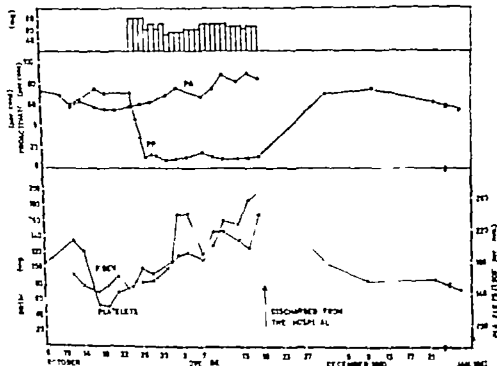


Fig. 11. Normalization of fibrinogen, proactivator and platelet count during anticoagulant treatment (phenylindanedione P. I. D.) in patient with giant haemangioma.

2. *New born babies*

Blood from the umbilical cord of ten new born babies was collected immediately after delivery and the plasmas examined. The proactivator concentration was low, the average value only 44 per cent of the concentration in plasmas from normal adults (table VIII).

3. *Pregnant women*

Blood from ten healthy women during the two last months of pregnancy was collected. The average proactivator concentration in plasma was 144 per cent (table VIII, fig. 10).

B. PROACTIVATOR DETERMINATION IN PLASMA FROM PATIENTS

1. *Postoperative patients*

The pregnant women had high proactivator levels in plasma and also high fibrinogen values (table VIII). Therefore, proactivator concentration was determined in plasmas from other patients with high fibrinogen level. Blood samples from ten patients 3—5 days after surgery were collected. The average fibrinogen concentration in the plasmas was much higher than in the pregnant women's, but the proactivator concentration was lower, though still higher than normal (table VIII, fig. 10).

2. *Patients with acquired fibrinogenopenia*

A patient with giant haemangioma, thrombocytopenia, fibrinogenopenia and fibrinolytic activity has previously been reported (Blix & Aas 1961). The patients fibrinogen value and platelets rose to normal during anticoagulant treatment, suggesting an intravascular coagulation process as the cause of the low fibrinogen concentration and platelet count. The patient was admitted to the hospital half a year later and the investigation was repeated with practically the same results (fig. 11). In the same figure the results of pro-

Table VIII Proactivator and fibrinogen values in plasma from normals and patients

Subjects (No.)	Fibrinogen (mg %)	Proactivator (%)
Normals (20)	268 (179—354)	100 (82—120)
Newborn (10)	182 (126—247)	44 (33—61)
Pregnant women (10)	437 (354—553)	144 (124—179)
Postoperative patients (10)	717 (580—902)	129 (106—147)
Anticoagulant patients (20)		115 (92—148)
Liver fibrosis (15) (Laennec's cirrhosis and chronic hepatitis)	261 (203—386)	62 (38—81)

The 100 per cent value in this study was defined as the average proactivator concentration in plasma from 20 normal subjects between 20 to 40 years of age (in plasma from ten women 101.7 and from ten men 98.3 per cent). The proactivator concentration in the plasma used as standard was 105 per cent. Each plasma tested was first assigned a value for per cent proactivator from the standard curve and finally expressed as per cent of normal. The range of proactivator in the twenty normal subjects was 82—120 per cent (table VIII). A standard deviation in the normal material of 10.8 per cent was calculated.

Every morning during five consecutive days the proactivator concentration in plasma from four healthy subject was tested. The variations from day to day were not significant (fig. 8). Three subjects were examined during 24 hours in bed (fig. 9) and the proactivator fluctuations were also insignificant. Plasma from two subjects after a fatty meal or excercise revealed no changes in proactivator levels.

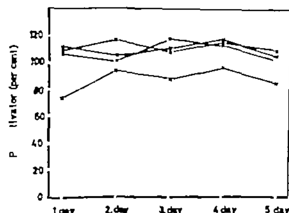


Fig. 8. Proactivator concentration in plasma from four healthy subjects every morning during five days.

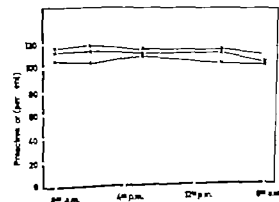


Fig. 9. Proactivator concentration in plasma from three healthy subjects during 24 hours.

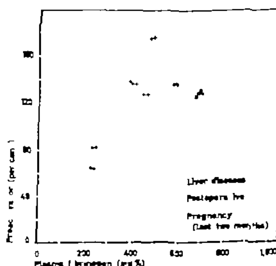


Fig. 10. Correlation between proactivator and fibrinogen concentrations in plasma from patients with liver diseases, patients 3—5 days after surgery and pregnant women.

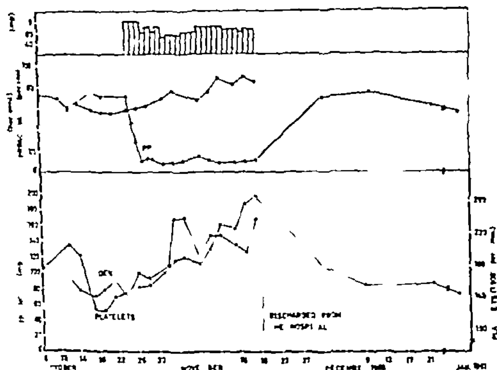


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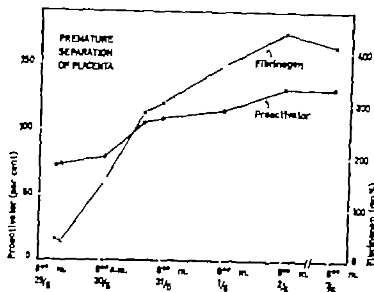


Fig 12. Premature separation of placenta a short time prior to the first blood examination.

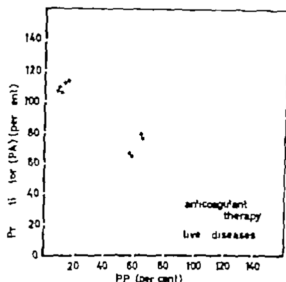


Fig 13. Correlation between proactivator and proconvertin-prothrombin per cent in plasma from 20 patients on long term anticoagulant treatment and 26 patients with liver diseases. Proactivator concentration was not depressed during anticoagulant treatment, while both proactivator and proconvertin prothrombin per cent were below normal range in the 15 patients who suffered from liver fibrosis (Laennec's cirrhosis and chronic hepatitis) (see text)

activator determinations in plasma are given. The proactivator concentration increased from about 60 to about 90 per cent.

b) Many investigators hav accepted that fibrinogenopenia following premature separation of placenta is due to a clotting process (Sherry 1959) We have had the opportunity to study such a patient and the results of the fibrinogen and proactivator determinations are given in fig 12 No increased fibrinolytic activity was found in the plasma samples.

The low proactivator concentration in these two patients may be explained by a clotting process as a great part of proactivator disappears from plasma during coagulation (table VI)

3 *Patients on anticoagulant treatment and patients with bleeding disorders*

Plasmas from twenty patients on long term anticoagulant treatment for thrombotic disorders have been examined, and the average proactivator concentration was slightly higher than in normal subjects (table VIII) i. e., proactivator is not depressed during anticoagulant treatment

Plasmas from patients with congenital afibrinogenemia, with severe haemophilia A, B and C, and with proconvertin and proaccelerin deficiencies were also examined, and the proactivator concentrations were normal.

4 *Patients with liver diseases*

Blood from 26 patients with liver diseases has been examined. The patients were admitted to the two medical departments of the University Hospital and to the six medical departments

Table IX. Proactivator determination in plasma from 26 patients with liver diseases performed with 6 days interval

Patients (No.)	Proactivator (per cent)	
	May 24th	May 30th
Liver fibrosis		
(Laennec's cirrhosis and chronic hepatitis)		
1	82	76
2	84	78
3	74	74
4	51	52
5	64	64
6	53	53
7	43	48
8	47	43
9	38	
10	64	
11	81	94
12	77	76
13		54
14		48
15	81	81
Acute hepatitis		
16	72	62
17	63	66
18		63
19	102	120
20	118	113
21	131	129
Unclassified		
22	128	127
23	120	113
24	122	127
25	149	133
26	113	

of the Oslo City hospitals. Blood from twenty of the patients was collected twice with an interval of six days. The material included 15 patients with liver fibrosis (Laennec's cirrhosis and chronic hepatitis) 6 patients with acute hepatitis and 5 patients with as yet unclassified liver diseases (most of them probably occlusions of the biliar duct). The proactivator concentrations in plasma of the 26 patients are reported in table VIII and IX. All the patients with liver fibrosis had abnormal low values. Table X gives the plasma fibrinolytic activity as tested on standard fibrin plates. Fig. 13 shows the correlation between the PP per cent and the proactivator per cent, and in fig. 10 the correlation between fibrinogen concentration and proactivator per cent is reported. (None of the patients with unclassified liver diseases had PP per cent below normal.)

C. PROACTIVATOR DETERMINATION AS A POSSIBLE LIVER FUNCTION TEST

The linear relationship between proactivator per cent and PP per cent indicates that proactivator determination may be used as liver function test in patients with liver diseases. However as the patients in this report were admitted to several medical departments an analytical and comparative study of the various liver function tests was difficult.

In 11 of the 15 patients with liver fibrosis electrophoresis was performed at approximately the same time as our proactivator determinations. Fig. 14 and 15 show the correlation between proactivator, albumin and γ-globulin concentrations in these plasmas. All factors were outside normal range. However no linear relationship between proactivator per cent and these protein fractions of plasma was found.

Table X. Fibrinolytic activity in plasma from patients with liver diseases, as tested on standard fibrin plates

Patients (No.)	Fibrinolytic activity				
	0	(+)	+	++	+++
Liver fibrosis (Laennec' cirrhosis and chronic hepatitis) (15)	5	5	3		2
Acute hepatitis (6)	3	2	1		
Unclassified (5)	5				

0: no fibrinolytic activity (normal). (+): traces of fibrinolytic activity (normal)
+: moderate fibrinolytic activity ++: distinct fibrinolytic activity +++: strong fibrinolytic activity

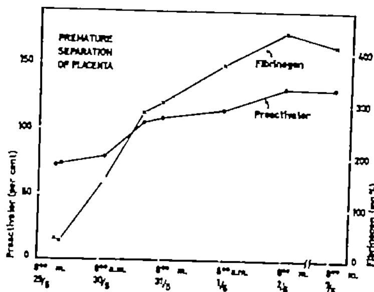


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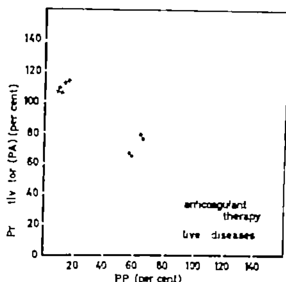


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3	74	74
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5	64	64
6	53	53
7	43	48
8	47	43
9	58	
10	64	
11	81	94
12	77	76
13		54
14		48
15	81	81
Acute hepatitis		
16	72	62
17	63	66
18		63
19	102	120
20	118	113
21	151	129
Unclassified		
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Unclassified (5)	5				

0: no fibrinolytic activity (normal) (+): traces of fibrinolytic activity (normal)
+: moderate fibrinolytic activity ++: [illegible]

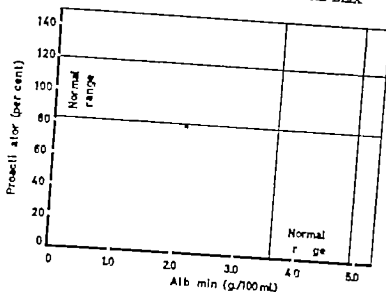


Fig 14 Correlation between activator and albumin concentr in plasma from 11 patients liver fibrosis (Laennec s cirrhosis chronic hepatitis)

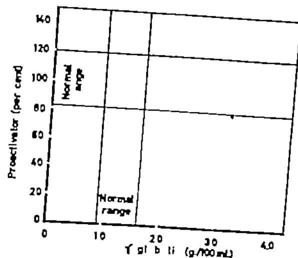


Fig 15 Correlation between proactivator and γ-globulin concentration in plasma from 11 patients with liver fibrosis (Laennec cirrhosis and chronl hepatitis)

Discussion

The system used in this study for proactivator determination in human plasma is based upon well known principles (Til lett & Garner 1933 Christensen 1949 Müllertz 1955 Blombäck et al. 1955 Nilsson et al. 1957 Lassen 1958) The method is accurate, reproducible and easy to per form.

Blombäck et al. (1955) have describ low 'plasminogen proactivator' concen trations in plasmas from three patien with haemorrhage and fibrinolysis assoc ated with surgery and Nilsson et al. (1955 found the same factors depressed in pa tients during extracorporeal circulation n association with cardiac surgery In 1960 Bergström et al. reported a case of liver cirrhosis with fibrinolysis and a proacti vator level in plasma of 25—50 per cent of normal.

We found a low concentration of proactivator in plasma from patients with impaired liver function (fibrosis) suggesting that proactivator is produced in the liver Proactivator was not depressed during anticoagulant treatment. A fairly good correlation between proactivator and fibrinogen concentrations in plasma could be demonstrated. Liver cirrhosis is often associated with increased fibrinolytic activity (Goodpasture 1914 Ratnoff 1949 Kwaan et al. 1956 de Nicola & Soardi 1958 Gross et al. 1961) We found increased fibrinolytic activity in some of our patients with liver fibrosis, but several with a low proactivator level had no increase in activity suggesting that the

fibrinolytic activity is not responsible for the proactivator deficiency (through a consumption of proactivator)

In some cases, a low proactivator concentration in plasma may be caused by a coagulation process. In vitro, we found that about 65 per cent of the proactivator content in plasma was present in serum. In a patient with a possible intravascular coagulation, the proactivator concentration increased to normal in parallel with the increase in fibrinogen concentration during anticoagulant treatment. Also in a patient with premature separation of placenta and acute fibrinogenopenia perhaps due to a coagulation process, the proactivator increased from low concentration to normal simultaneously with the rise in fibrinogen level.

In the present study 26 patients with liver diseases were examined with respect to proactivator PP and fibrinogen concentration in plasma. The material comprised 15 patients with liver fibrosis (Laennec's cirrhosis and chronic hepatitis) 6 patients with acute hepatitis and 5 patients with as yet unclassified disease (probably mainly suffering from occlusive biliary disease, but none of them had a PP per cent below normal value when the proactivator determinations were performed) The linear relationship between proactivator and PP per cent, and the limited correlation between proactivator per cent and fibrinogen concentration, suggested that proactivator determination in plasma might be a valuable supplement to other liver function tests, the more so as the proactivator was not depressed during anticoagulant treatment. In 11 of the 15 patients with liver fibrosis a comparison between proactivator albumin and γ-globulin concentrations in plasma was possible, and all values were abnormal. Further investigations and a great number of patients are required in order to study the clinical value of proactivator determination.

Summary

1 A modified method for the determination of proactivator concentration in human plasma has been described

2 Proactivator concentration in serum was about 65 per cent of that in plasma. Up to 68 per cent of the proactivator content could be precipitated with the euglobulin fraction of plasma.

3 The normal range of proactivator concentrations was 82—120 per cent. The diurnal fluctuations and the variations in proactivator concentrations in plasma from day to day were insignificant. Proactivator concentration was low in plasma from newborn babies and patients with impaired liver function high in plasma from pregnant women and postoperative patients, and normal in plasma from patients on anticoagulant treatment and patients with congenital bleeding disorders, including afibrinogenemia.

4 During anticoagulant treatment of a patient with giant haemangioma and a possible intravascular coagulation, the low proactivator concentration in plasma rose to normal. In a patient with premature separation of placenta a low proactivator level was recorded but the concentration increased simultaneously with the rise in fibrinogen concentration.

5 A linear relationship between PP per cent and proactivator per cent in patients with liver diseases has been demonstrated. Fifteen patients with liver fibrosis (Laennec cirrhosis and chronic hepatitis) had an abnormal low proactivator concentration in plasma, and the possibility of using proactivator determination as a liver function test has been discussed.

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Radioiodine Thyroid Scanning as Applied to Thyroid Problems in an Endemic Goitre Region

By

G Hintze, B. A. Lamberg P Wahlberg and P Malm

Nodules in the thyroid or in the neck always give rise to diagnostic problems. A new approach was made with the introduction of the "scanning" or "mapping" of the thyroid region with radioactive iodine (7) Since then this method has been used more or less as a routine examination for the evaluation of the functional state of thyroid nodules especially after the introduction of the automatic scanning device of Allen and co-workers (1 2, 3 4) It was soon observed that the presence of hyperactivity in a thyroid nodule as compared with the surrounding tissue almost always excluded the possibility of this nodule being thyroid carcinoma. In contrast, hypoactive nodules are very often thyroid carcinomas and cancer may also be present in a number of normoactive nodules.

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In Finland nodular goitre is prevalent in from 65 to over 90 per cent of the patients with goitre (14 17 25 44) Multinodular goitre is fairly frequent, which creates certain diagnostic problems. Hence it seemed to be of interest to find out to what extent thyroid scanning may be used for diagnostic purposes in such a material. Since 1955 thyroid scanning has been practised at the IVth Medical Clinic and the Maria Hospital, and later at the Minerva Institute for Medical Research. The present report presents the experience gained during the last 6 years. A few of the cases included here have been reported in a previous communication (24)

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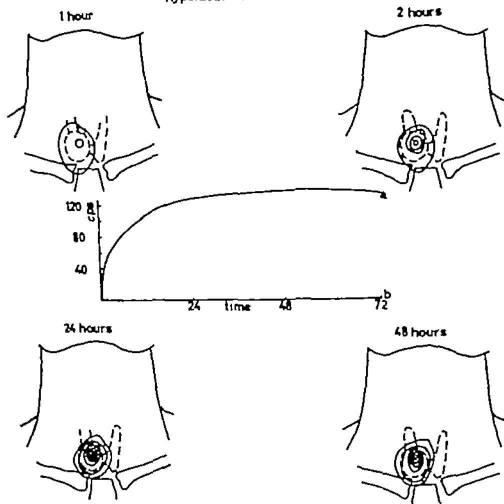


Fig. 1 Serial scanning of "hot" nodule. The uptake by the surrounding tissue is virtually nil. Curve a in the diagram in the middle of the picture represents the uptake by the nodule, curve *b* that of the paranodular tissue. The solid lines depict the radioactive area. The difference in uptake between the nodule and the surrounding tissue is established within a few hours after the dose.

by the paranodular tissue was virtually nil, and it was noticed how rapidly the difference in collecting ability between the nodule and the paranodular tissue was established. Already within a few hours the difference was quite distinct.

No thyroid carcinoma was detected in this group of patients.

In 11 cases the thyroid nodule was *normoactive* i. e. there was no difference between the uptake by the nodule and that of the surrounding tissue. The nodule was removed in 6 cases. In 4 of them the nodule was a colloid one, in 1 case a true adenoma. In the sixth one, papillary thyroid carcinoma was detected. Of the

Material and methods

All together 131 patients have been scanned, of whom 120 are included in this report. The reason for carrying out scanning of the thyroid region has been mainly 1) the presence of a solitary nodule in the thyroid, 2) the appearance of a "solitary" nodule in multinodular goitre as defined by Malm (28) meaning a nodule which differs with regard to growth, consistency and other clinical signs from other nodules in the same gland, 3) the appearance of nodules in the thyroid remnants after surgical treatment for hyperthyroidism or non-toxic goitre, 4) check up examination of the thyroid remnants after radical surgical treatment for thyroid carcinoma, 5) some cases of subacute thyroiditis.

The patients were referred to the isotope laboratory mainly from the wards of the IVth Medical Department and the Maria Hospital and the Endocrine Out Patients Department partly from the 1st Medical Clinic, the Eira Hospital and other hospitals.

The patients were given 20 to 250 μC of radioactive iodine (^{131}I Amersham, England) orally and the thyroid scanning was carried out usually either 24 or 48 hours later In 12 patients serial mapping was carried out 1 2 4 24 48 and 72 hours after the administration of the dose.

The scanning was carried out using a manual method and the procedure has been *somewhat changed* during the years. In earlier years a plastic sheet with a chequered pattern drawn in Indian ink, the squares of which had a width of 18 × 18 mm was placed on the neck of the patient, and the counting rate recorded above each square (24) Later when a ratemeter was used, the procedure was simplified, and during the last years the counting rate was continuously recorded as the detector was pushed by hand over the area to be studied. The collimation equipment has also been modified during the years. To start with, the cone-shaped small collimator of Ekco was used, but later a more efficient one was made of lead according to the principles described by Jensen (17) The gamma-scintillation detector was provided by Ekco (Ekco Electronics Ltd England Type N 50 B)

Table I Presentation of the cases in which scanning with radioactive iodine was carried out

Solitary nodules	
Hyperactive	20
Normoactive	11
Hypoactive	25
Multinodular goitre	10
Postoperative states	28
Extrathyroid nodules	4
Intrathoracic problems	16
Subacute thyroiditis	6
	Total 120

Includes 2 cases previously operated upon for thyroid carcinoma.

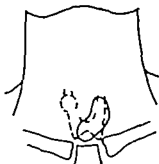
Results

The results are presented in tables I to IV Some typical scanning maps are presented in figs. 1 to 8 In table I is shown the distribution of the patients according to the type of problem studied.

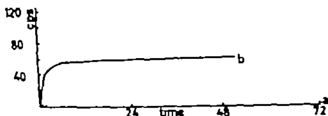
1 *Solitary nodules*

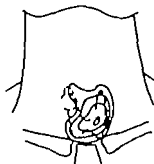
There were 20 *hyperactive* nodules, of which 6 were toxic and the remaining "hot" with euthyroidism In the toxic cases the paranodular tissue collected only very minute amounts of radioactive iodine, whereas in the remaining cases the paranodular tissue collected relatively more All of the toxic nodules were surgically removed and the diagnosis of *toxic adenoma was confirmed histologically* Out of the 14 "hot" nodules 7 were removed operatively In 6 of these the histological examination revealed the presence of a true adenoma whereas in the seventh one the nodule consisted only of hyperplastic thyroid tissue. In a few of the cases belonging to this group serial scanning was carried out The observations made in one of these cases are depicted in fig 1 In this case the uptake

Hypoactive nodule









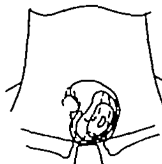


Fig 2. Serial scanning of cold" nodule. Curve *b* represents the uptake by the surrounding tissues, curve that of the nodule The difference in uptake is evident already after few hours. The solid line represent the radioactiv area.

a few hours after the dose. This will have some practical importance because, as was seen in some of our cases, when the hypoactive nodule is small in size the hypoactivity may be overshadowed by the radiation from the normal tissue, t least with the method used by the authors.

2. *Multinodular goitre*

Scanning was carried out in only 10 cases with a multinodular goitre. With this method it was possible to confirm the heterogenity of the intrathyroid iodine metabolism which, partly t least, is seen in the histological picture (43) The findings in one of these cases are shown in

Table II The distribution of radioactivity in the thyroid and the ultimate diagnosis in patients with a single thyroid nodule and with multinodular goitre

Diagnosis	No. of subjects	Euthyroid	Hyper thyroid	Uptake of the surrounding tissue		No. of patients operated upon	Ultimate diagnosis
				−	+		
1 Hyperactive nodule	20						
— Toxic nodule	6	—	6	5	1	6	Toxic adenoma 6
— "Hot" nodule	14	14	—	7	7	7	Adenoma 6, nodular goitre 1
2. Normoactive nodule	11	11	—	—	11	6	Adenoma 1 nodular goitre 4, papillary carcinoma 1
3 Hypoactive nodule	25	23	2	—	—	21	Nodular goitre 9, adenoma 3, embryonal adenoma 1 foetal adenoma 2, cyst 3 different carcinoma 3
4 Multinodular goitre	10	10	—	Variable		8	Nodular goitre 5, adenoma 2, embryonal adenoma 1

remaining cases, one was treated with desiccated thyroid and 4 patients have not been seen at the follow up examinations. The thyroid treatment induced a decrease of the size of the nodule, suggesting that it was not an adenoma.

In 25 cases the nodule was *hypo-active* It is quite natural that a larger percentage of these nodules was surgically treated The ultimate diagnosis in the 21 cases undergoing operation appears in table II In 9 cases presenting a non functioning colloid goitre nodule, two of the patients had in addition Graves disease. In 6 cases, there was a true non functioning adenoma of varying histological type In 3 cases, there was a cyst, and one of these patients also had Graves disease. It is noticeable that in 3 patients out of the 21 that were operated upon, thyroid carcinoma was found The carcinoma was of the follicular or mixed follicular papillary type.

In 3 patients treatment with desiccated thyroid was instituted and the thyroid nodule decreased in size. One patient was not treated at the time of study

Fig 2 depicts the findings at serial scanning in a case of hypo-active nodule. Here again it was noticed that the difference in collecting ability between the hypoactive nodule and the surrounding tissue was fairly well established already

fig 3. In 8 cases subtotal thyroidectomy was performed, and in 3 of these cases true adenomata were found. In the rest of the patients the nodules consisted only of colloid goitre.

3. *Postoperative states*

This group comprises 28 cases in which thyroidectomy had been carried out previously In a few of them scanning was made only to ascertain whether there was any functioning tissue left. The "radioiodine maps" of two cases with thyroid carcinoma and regional metastases are shown in fig 4 and 5. Both cases illustrate the collecting ability of the thyroid remnants left at operation, but in fig 5 one may also observe the presence of thyroid tissue with fairly high uptake laterally in the neck, evidently consisting of stimulated functioning metastases. Although the metastases histologically were of the functioning type, the collection of radioactive iodine by them was too small to be detected by the scanning method used.

The main reason for carrying out thyroid scanning postoperatively has been the appearance of nodules in the thyroid remnants, the postoperative development of a nodule in the midline of the neck outside the true thyroid region, and the appearance of an intrathoracic mass.

The situation here designated as solitary nodule has been created either by a single nodule ppearing in a hypertrophied thyroid remnant or a very small thyroid remnant appearing as a hard nodule. Such nodules" have usually ery hard consistency which may create suspicion of thyroid carcinoma. The logical explanation for such a situation is, of course, the postoperatively occurring compensatory TSH-induced hypertrophy which, in some cases, may

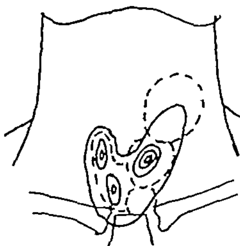


Fig. 3. Scanning of multinodular non-toxic goitre showing the heterogenity of the distribution of radioactivity within the gland. The solid lines represent the radioactive area.

possibly ppear more prominently in some parts of the actively stimulated thyroid remnant. There were 12 cases belonging to this group, and in 9 of them presence of a very high uptake in the nodule could be confirmed. In some of the cases, the suspicion of a very fast intrathyroid turn-over of radioiodine was confirmed for instance by determination of the organic plasma radioactivity In one case there was recurrent hyperthyroidism. In another case a cyst was found on operation. In those cases that had a recurrent multinodular goitre, again the heterogenity of the thyroid function could be noticed. In fig 6 the result of the scanning in a case with

Table III The distribution of radioactivity in the thyroid and the ultimate diagnosis in some cases presenting various problems after previous thyroidectomy

Clinical diagnosis	No. of subjects	Result of scanning	Operation	Ultimate diagnosis	Treatment with desiccated thyroid	
					No change in size	Decrease in size
1 Thyroid remnant presenting as a nodule	2	Activity 2	—	—	—	1
2 Solitary nodule in hypertrophic remnants	12	Hyperactive nodule 9	—	—	1	5
		Normoactive nodule 1	1	Diff carcinoma 1	—	—
		Hypo-active nodule 2	2	Diff carcinoma 1	—	—
				Cyst 1	—	—
3. Multinodular postoperative hypertrophy	7	Varying Activity 7	—	—	1	4
4. Intrathoracic tumour	4	Activity 4	2	Nodular goitre 2	—	—
5 Tumour in the neck	3	Activity 3	2	Nodular goitre 2	—	1

Table IV Result of scanning with radioactive iodine in 16 cases with intrathoracic problems and 4 cases with a tumour in the neck

Diagnosis	No. of subjects	Operation	Final diagnosis
Intrathoracic tumour	16		
Uptake	10	8	Nodular goitre 8
No uptake	6	6	Aortic aneurysm 1
			Aneurysm of the innominate artery 1
			Metast. oesophageal carcinoma 1
			Non-defined tumour (not operat.) 1
			Lymphadenitis, tuberculous 1
			Pulmonary tuberculosis or carcinoma 1
Extrathyroid nodule			
No uptak	4	4	Branchial cyst 1
			Dermoid cyst 1
			Tuberculous lymph node 1
			Pulmonary carcinoma with generalized metast. 1

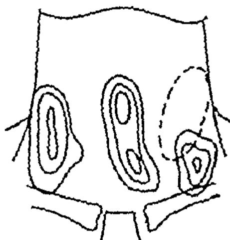


Fig. 3. Scanning in a case with metastasizing thyroid carcinoma (papillary type). The palpable tumour did not collect any radioactive iodine but radioactivity was detected at the site of the thyroid remnant left after operation and also laterally in the neck indicating the presence of functioning metastases which were not palpable.

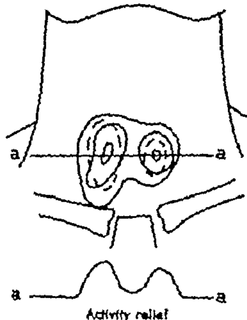


Fig. 6. Scanning of postoperatively hypertrophic thyroid remnants presenting as two hard tumours on both sides of the neck.

case was tuberculosis of the lymph nodes and in the latter case metastasizing pulmonary carcinoma. In two cases the nodule appeared higher in the neck quite apart from the thyroid but in the midline suggesting that this nodule could be the remnant of the thyroglossal duct. In one of these cases the final diagnosis was a branchial cyst, and, in the other a dermoid cyst.

5. *Intrathoracic masses*

In addition to those postoperative cases mentioned above, there were 16 cases in which the presence of a mediastinal mass caused some diagnostic trouble. In 10 of these cases, as well as in the 5 previously operated upon (group 3), the radioiodine scanning revealed the presence of radioiodine at the site of the intrathoracic mass. Several of these cases were operated on, and the diagnosis of intrathoracic non-toxic nodular goitre was confirmed on histological examination. One illustrating case record may be shortly reviewed.

Male, 44 years, 11 years previously treated surgically for hyperthyroidism. During the last 8 years the patient became increasingly short of breath. Several X-ray examinations were made during these years, and in all a large mediastinal mass was detected. The nature of this mass was, of course, open for discussion and speculation, and among other X-ray diagnoses the following were made: 1) neurinoma, hardly intrathoracic goitre; 2) cannot be thyroid tissue, probably of lymphatic origin. Lymphoma. 3) mediastinal tumour. Lymphoma? Goitre? 4) not an aneurysm, probably goitre. 5) cannot be thyroid tissue as the mass is situated behind the trachea.

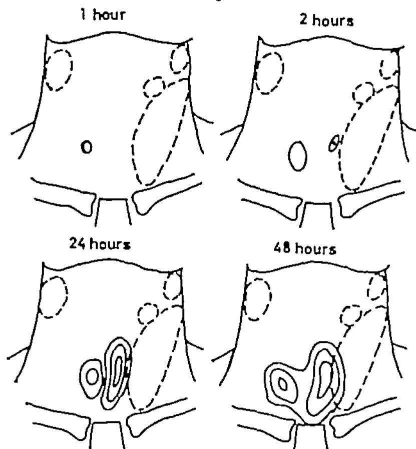


Fig 4 Serial scanning in a case of metastasizing thyroid carcinoma (alveolar type) Radioactivity was detected only at the site of the thyroid remnants left at the thyroidectomy Solid lines represent the radioactive area.

very hard nodule like hypertrophied thyroid remnants is presented.

In 3 cases, there was a postoperative development of a nodule higher in the neck above the thyroid area. These nodules appeared in the midline area, and in all 3 cases there was a small or a hypertrophied remnant left in the thyroid region. In all these cases, the nodule higher in the neck collected radioactive iodine in two of them in appreciably large quantities, and in one of them the diagnosis of hyperfunctioning thyroid tissue was confirmed after surgery The scanning map found in this case is shown in fig 7 In the third case, the extra thyroid nodule collected only small amounts of radioactive iodine, and at surgical exploration a papillary carcinoma developing in the wall of a thyroglossal cyst was found.

In 4 cases previously treated with thyroidectomy an intrathoracic mass appeared later on, which was demonstrated by X ray examinations. The practical problems in such cases are the same as those usually arising in patients with intrathoracic masses (see below)

4 *Palpable masses close to the thyroid gland*

In 2 cases there was a fairly large mass palpable close to the thyroid area or situated so that the relationship to the thyroid gland could not be evaluated with certainty In both cases the palpable mass did not collect radioiodine which excluded the presence of functioning thyroid tissue. The diagnosis in the former

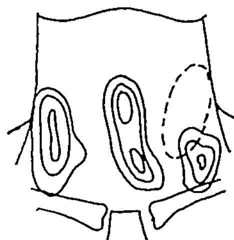


Fig. 5. Scanning in case with metastasizing thyroid carcinoma (papillary type). The palpable tumour did not collect any radioactive iodine but radioactivity was detected at the site of the thyroid remnants left after operation and also laterally in the neck indicating the presence of functioning metastases which were not palpable.

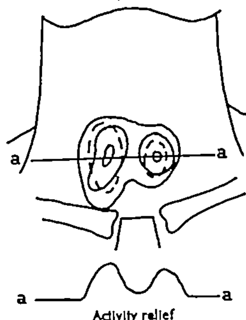


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In addition to those postoperative cases mentioned above, there were 16 cases in which the presence of a mediastinal mass caused some diagnostic trouble. In 10 of these cases, as well as in the 3 previously operated upon (group 3) the radioiodine scanning revealed the presence of radioiodine at the site of the intrathoracic mass. Several of these cases were operated on and the diagnosis of intra thoracic non-toxic nodular goitre was confirmed on histological examination. One illustrating case record may be shortly reviewed

Male, 44 years, 11 years previously treated surgically for hyperthyroidism. During the last 8 years the patient became increasingly short of breath. Several X-ray examinations were made during these years, and in all a large mediastinal mass was detected. The nature of this mass was, of course, open for discussion and speculation, and among other X-ray diagnoses the following were made 1) neurinoma, hardly intrathoracic goitre 2) cannot be thyroid tissue, probably of lymphatic origin. Lymphoma 3) mediastinal tumour Lymphoma? Goitre? 4) not an aneurysm, probably goitre 5) cannot be thyroid tissue as the mass is situated behind the trachea.

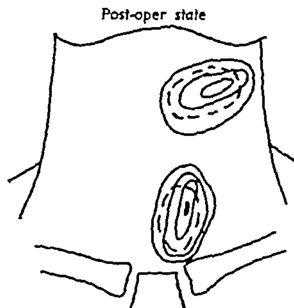


Fig. 7 Scanning of postoperative hypertrophic remnants presenting as two hard tumours, one at the original sit of the left lobe one higher up in the neck in the mid-line area. An appreciable uptake was bserved in both tumours indicating these to be hypertrophic remnants. The higher tumour was removed surgically and the clinical diagnosis histologically confirmed Evidently the tumour was a thyroglossal remnant.

The importance of radioiodine scanning in cases of this kind is quite obvious. In the case reported scanning revealed the presence of functioning thyroid tissue, and a large intrathoracic goitre was removed surgically

In 6 cases no intrathoracic collection of radioiodine could be detected. On surgical exploration in 3 of them no thyroid tissue was found and the diagnoses were aortic aneurysm, aneurysm of the innominate artery metastasizing oesophageal carcinoma. In the three other cases the ultimate clinical diagnosis was not one of thyroid disorder

6 *Subacute thyroiditis*

It is well known that the thyroid uptake of radioactive iodine is very low in some cases almost nil, in the acute phase of subacute thyroiditis (6 21 42) The distribution of radioactive iodine within the thyroid may however vary This is

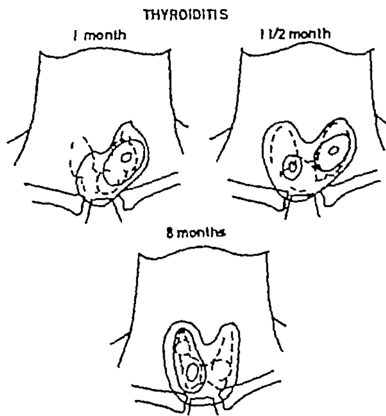


Fig 8 Scanning in a case of subacute thyroiditis at different times after th cut attack. The changing uptake pattern may be noted.

especially true in the state of regeneration observed in several of our own cases (21) On the other hand subacute thyroiditis may appear as a focal or a migrating condition (5, 27) In such cases it will be possible to observe the development of the disease when the thyroid is scanned at certain intervals (45) One typical case is shown in fig 8. In the acute phase the diseased part of the thyroid is clearly hypoactive as compared with the rest of the thyroid. 1½ months later at the stage of temporary hypothyroidism, the uptake increases and the distribution of the radioactivity seems to be fairly even within the thyroid. Eight months after the acute phase at the stage of regeneration the thyroid uptake is increased above normal, and this is especially true with regard to the originally diseased part of the thyroid. At this stage that part exhibits a higher uptake than does the remaining part of the thyroid.

Discussion

The authors are aware of the fact that the device used by them for thyroid scanning is not adequate for the distinction of a small area within the thyroid with a small increase or decrease of the uptake of radioactive iodine as compared with the surrounding tissue. During recent years, various electronic contrivances have been constructed in order to increase the resolving power of the scanning device (10) The use also of special collimation arrangements may increase the discriminating power (40) It is, however evident from the present results that, even with a very simple manual technique like the one described here, scanning will provide much valuable information which could not be gained easily in any other way

From a practical point of view thyroid scanning seems to be of appreciable importance in Finland where the primarily iodine deficient goitre (16 22, 23 41) is characterized by a high incidence of nodules. Hence, the solitary nodules, for instance, will create problems which do not exist in a non-goitrous district. It is to be expected that there are hyperactive nodules which do not have the histological structure of a true adenoma but only represent an initial hyperplastic and hyperactive stage in those events that ultimately result in nodular goitre (38, 39) In one of the hyperactive nodules in the present series, for instance, this proved to be true as evidenced by histological studies and studies of the kinetics of the intrathyroid iodine metabolism. Among the cases studied in Finland during the last few years some cases have been found which evidently represent the thyroid two-compartment model first suggested by Stanbury and collaborators in their studies in the Andes (35) It is more than possible that the existence of such hyperactive nodules even when they are not true adenomata may favour the development of Plummer disease and Jod Basedow (18) Whereas thyroid treatment does not affect the true adenomata in any way (8 32, 33) this treatment may however have some beneficial effect in cases of the type mentioned (9 29 30) The results of thyroid treatment in some of the cases presented here and in some cases previously reported (20) are in favour of these views. Perlmutter and Slater (30) reported that in about half of their cases the hot nodule failed to be inhibited by thyroid treatment.

With regard to the use of radioiodine scanning in connection with hypoactive nodules the situation seems to be very complex. As might be expected there

were several cases in which the ultimate diagnosis was that of a cyst benign adenoma or colloid nodule. In fact the colloid nodules represented about 50 per cent of the cases, which is not very surprising in an endemic goitre region But the value of the scanning procedure is established also in the case of the hypoactive and the normoactive nodule as about 14 per cent of the cases proved to have a differentiated thyroid carcinoma This incidence is of the same magnitude as reported from other sources (11 26 31, 34)

One more thing which to the authors seems to be of great importance with regard to the conditions prevailing in Finland is postoperative tissue hypertrophy It must, of course, be borne in mind that the causes of endemic goitre will not be eliminated by thyroid surgery Hence, a so-called relapse is almost a requirement for persisting euthyroidism in an endemic goitre region although only a certain percentage will call for therapeutic measures some 10 to 15 years afterwards (36) As mentioned already in the previous section in cases where the thyroid remnants are small and hard, thus resembling a single nodule or a thyroid carcinoma as well as in cases where there is a solitary nodule within the hypertrophied remnant scanning with radioiodine will give an almost certain answer as to the nature of the situation at hand. Especially valuable is the combination of scanning and an analysis of the kinetics of the thyroid iodine metabolism by analysing for instance the thyroid ^{131}I retention curve and determining the plasma protein bound radioactivity (15 18) In such cases treatment with thyroid hormones or desiccated thyroid starting soon after operation, would seem to be of fairly great importance (15) In fact such a problem arises almost every day owing to the fact that no less than some 1,500 to 2 000 patients are thyroidectomized every year for non-toxic endemic goitre (13 44) The small thyroid remnants, sometimes only of the size of a finger tip will not always even be able to produce the required amount of thyroid hormones in spite of a very fast intrathyroid turn-over (18) Such cases will create situations troubling the patient and also the physician, if he is not familiar with the basic physiological aspects involved. A thyroid nodule of this kind should of course, not be removed for fear of thyroid carcinoma or for cosmetical reasons, as is often done, but should be treated with thyroid hormones. For making this decision thyroid scanning may be of importance, although it must be admitted that it is not always an absolute requirement.

It must be observed also, that in the endemic goitre in Finland intrathoracic goitre is frequently found (17 37) Without the help of radioiodine scanning the nature of such intrathoracic masses will always be open for speculation as may be clearly seen in many of the cases here presented By using radioiodine scanning the problem will certainly be much simplified

To what extent thyroid scanning would be important when planning, for instance treatment with radioactive iodine for toxic nodular goitre is, so far not apparent It must however be borne in mind that the largest group of hyperthyroidism requiring treatment with radioactive iodine in Finland is undoubtedly the one with nodular goitre (19) This applies of course mainly to people in the older age group but the functional heterogeneity within the thyroid and the presence of different intrathyroidal io-

dine compartments with different turnover rates, is a factor which may affect the dosage and calculations. It may be worth while to pursue this matter further.

Summary

Scanning of the thyroid area, and in certain cases of adjacent regions, was carried out in 120 patients presenting various thyroid problems after a test dose of ^{131}I. A solitary nodule was present in 56 patients, and of these 20 were hyperactive, 11 were normoactive, and 25 hypoactive. The hyperactive nodule may represent a true hyperfunctioning adenoma or only a focal hyperplasia with accelerated intrathyroid iodine metabolism, and may ultimately develop into Plummer's disease or undergo degenerative changes. Among the normo- and hypoactive nodules, almost 50 per cent consisted of colloid nodules, but in this group the incidence of differentiated thyroid carcinoma was 14 per cent. In some cases thyroid cysts and benign adenomata of various histological types were found.

In 10 cases with multinodular goitre the intrathyroid functional heterogenity was demonstrated by this method.

In 28 patients who had been thyroidectomized 2 to 10 years previously the appearance of hard nodules in the remnants had created some diagnostic problems. In most of the cases the thyroid scanning revealed very active thyroid tissue, evidently as result of a compensatory overstimulation by TSH. The diagnostic importance of thyroid scanning and kinetic studies of the intrathyroid iodine metabolism is emphasized in such cases, and the value of thyroid treatment has been discussed.

In 16 cases scanning with radioactive iodine revealed an intrathoracic goitre in 10 instances. The importance of this examination in cases of this kind is stressed.

On the whole thyroid scanning has wider applications in an area with endemic goitre than in a nongoitrous region. This applies especially to postoperative conditions and intrathoracic problems, both of them being fairly frequent in Finland. The same applies also to the solitary nodules in which radioiodine scanning very often reveals the presence of a hyperfunctioning area in the thyroid. With regard to the presence and detection of differentiated thyroid carcinoma the situation seems to be the same as reported in other studies, but in an endemic goitre region non functioning colloid nodules and degenerative nodular changes makes the problem somewhat more complex.

During the course of subacute thyroiditis, scanning of the thyroid in different stages will provide a view of the recovery of the thyroid function.

Acknowledgement

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Coagulability of Blood after Ingestion of a Fatty Meal

By

Kristoffer Korsan Bengtsen

Great attention has been paid to the possible role of food in the formation of intravascular thrombosis. One of the approaches to this problem has been to study the coagulation in vitro before and after a fatty meal. The main results of such studies have recently been reviewed (1 2) Most authors have demonstrated a shortening of the plasma clotting time in the presence of Russell viper venom (R. V. V.) 3—4 hours after a fatty meal (4 6, 9 11 12, 15 16 17 19 24 41 26 53) The results of the studies using whole-blood clotting time (W. B. C. T.) are more divergent. Many authors report a shortening also of the W. B. C. T. in silicone-treated tubes (5 7 8, 12, 13, 15 16 18, 26, 27 41 42, 45) and some authors report a shortening of the recalcification time (10 13 17 43) 1—4 hours after a fatty meal Other investigators have failed to demonstrate this effect when using the W. B. C. T. (4 19 20, 21 22, 23 24 25 44 53) A certain correlation between the increase in plasma lipids and the decrease of the clotting times has been observed by some investigators (26 27 9 41 54) while this correlation is denied by others (15 4 5 6 7 10 8) There are also divergent opinions on the importance of the degree of saturation of the fats given. In one work it was found that the increase on coagulability was higher after oral administration of unsaturated than after saturated fats (18) Conversely in other works the saturated fats seemed to be more active (8, 42) In most studies, however the degree of saturation was found to be of no importance (6 10 13 14)

The "sludgeing of blood" which appears under certain circumstances has been thought to promote the formation of thrombosis. In this condition the formed elements are trapped in the periphery and a reduction of cells would thus be expected in the circulation. A drop in the number of platelets in the circulating blood has been found after a fatty meal (15 39 40, 50) As the platelets during the coagulation of blood normally aggregate and un-

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and 900 mm in length, were filled w th blood sealed at both ends with plasticine and left at room temperature for 10 minutes. Then 5 mm of the capillaries was broken off every 30 seconds. The end point of the clotting time was taken when the blood could be drawn out as thread.

LIPID ANALYSES

Cholesterol analyses was made directly on citrated plasma by the method of Zlatkis, Zak, and Boyle (38)

Extraction procedure A portion of 5 ml of the citrated plasma was blown down into 100 ml of mixture of 2 parts of chloroform A. R. and one part of methanol A. R. at room temperature. After standing 1 hour at room temperature, the chloroform-methanol extract was filtered through a glass filter number 4 and evaporated to dryness under reduced pressure and t temperature of + 50° C. The residue was reextracted with 4 successive portions of chloroform of + 50° C. The chloroform extract was filtered through fat-free filter paper into measuring flask and adjusted to 25 ml at a temperature of +20° C. On this chloroform extract the total lipid and lipid phosphorus determinations were performed. All analyses were performed in duplicate.

Lipid phosphorus was determined by the method of Fiske and Subbarow (36) as modified by Brante (37)

Total lipids. Two 5 ml portions of the chloroform extract were evaporated and the residue was weighed (37).

The figures for the lipid concentrations express the content in citrated plasma thus the figures hav not been adjusted for dilution with the citrate solution.

STATISTICAL METHODS

1 The mean differences between the fasting alues and the values after 1—7 hours respectively in the cream and oliv oil groups were compared with the corresponding mean differences in the control group on the hypothesis of equality in changes in clotting times and lipid concentrations. Differences between two mean alues are thus analyzed with Student t-test.

$$t = \frac{X_1 - X_2}{\sqrt{\frac{S_1}{n_1} + \frac{S_2}{n_2}}}$$

Where X_1 is the mean difference in the cream or olive oil group, X_2 the mean difference in the control group, and S_1 and S_2 the corresponding variances, and n_1 and n_2 the corresponding numbers. Tables of the probability function of t then gives the probability of the observed difference.

2. The mean differences between the fasting values and the values after 1—7 hours on percentage basis, in the cream and olive oil groups were compared w th the corresponding percentage differences in the control group. The calculation was performed as abov Here X_1 is the mean of the differences on a percentage basis in the cream or olive groups and X_2 corresponding mean differences in the control group.

3 The correlation analyses were performed by ordinary statistical methods. Correlation coefficient =

Significance levels

0.05 ≥ P > 0.01 Significant
0.01 ≥ P > 0.001 Highly significant
0.001 ≥ P > Very higly significant

Material

Healthy young students or patients with neuritis of sciatic nerv or a peptic ulcer were used for the experiments. All subjects had been fasting for 13 hours before the experiment was started. Three groups wer investigated, one group of 14 subjects who wer given water only (control group) one group of 12 subjects who were given 3 ml of 40 cow' cream per kg body weight, and one group of 10 subjects who were given 1 ml of oliv oil per kg body weight.

Results

Th figures for whole blood clotting time, Russell's viper venom clotting time, lipid phosphorus, and t tal lipids are presented in table I. In table II and III the significance levels of the differences between the control group and the olive oil and cream groups are shown calculated both with the actual figures and on a per centage basis.

dergo "viscous methamorphosis" some kind of connection between the platelet drop and the increased coagulability of the blood has also been thought to exist. An increased fragility of the red cells has also been demonstrated after fat ingestion (46 47 48 50). Other authors have, however, not been able to verify this (19).

In spite of the many investigations on the effect of a fatty meal on the blood coagulation, the question is not definitely settled. It seems to be established that the Russell's viper venom recalcification time is shortened, but it is uncertain if this test reflects an increase in the tendency of the whole blood to clot. Indeed, no significant decrease of the whole blood clotting time was found in the study which seems to be the most thorough (55). It was, therefore, felt to be of value to publish the details of a study on this subject which was reported in preliminary form in 1956 (28). In this investigation the effect of oral administration of cream or olive oil was studied on healthy subjects and compared with the effect of water in a control group. Whole blood clotting time in silicone treated glass capillaries, prothrombin, prothrombin-proconvertin (PP), proaccelerin, recalcification time with Russell's viper venom, platelets, red blood corpuscles, Hb, total lipids, lipid phosphorus, and cholesterol were determined before and at hourly intervals after the meals.

Cream was found to decrease the whole blood clotting time and the recalcification time with Russell's viper venom, while olive oil gave a significant decrease only of the recalcification time with Russell's viper venom. There was no significant correlation between the effect on the coagulation and the increase in lipid concentrations. The number of circulating blood corpuscles was not significantly reduced.

Methods

Collection of blood

Venepunctures were made in the antecubital vein with minimal stasis, alternating between the two arms with sharp and coarse silicone treated needles. The first 2—3 ml of blood were discarded and then the blood was allowed to flow directly into silicone-treated glass centrifuge tubes. In the first tube, 1—2 ml were collected and from this sample the glass capillaries for the whole-blood clotting time (see below) were filled immediately. In the second tube, which was heparin-treated, 2—3 ml were collected. From this sample blood was immediately taken for the counting of platelets and red blood corpuscles and for the Hb analysis. In the third tube which contained 2.5 ml of 3 % trisodium citrate, 22.5 ml of blood were collected, immediately mixed, and centrifuged in an "International" refrigerated centrifuge model PM 2 at 1700 RCF and + 4° C for 30 minutes. The plasma was collected with siliconetreated pipettes into silicone treated glass tubes. On this plasma the remaining coagulation tests and the lipid analyses were performed. All the glass tubes for the collection of blood were kept in an ice bath before and after the collection of blood.

Haematological methods

Enumeration of platelets was performed with Brecher and Cronkite's method (34).

Enumeration of red cells was performed with Hallberg's method (35). By this method the cells were counted on a photograph of the central area of the counting chamber. With a red cell count of 4.0 millions per mm³ 3 000 cells were enumerated.

Proaccelerin. A modification of the method of Aas (29), now published (30), was used.

Prothrombin. The method of Hjort, Rapaport, and Owren (31).

Prothrombin-proconvertin (PP). The method of Owren and Aas (32).

Russell's viper venom time (R. V. V. T.). 0.2 ml of plasma was mixed with 0.2 ml of Russell's viper venom (Stypven, Burroughs Wellcome & Co., London, England) 1:10 000 and 0.2 ml $CaCl_2$ 0.025 M was blown down. The test was performed at 37° C.

Whole-blood clotting time (W. B. C. T.). A modification of the method of Mayer (33). Silicone-treated glass capillaries, 0.5 mm in diameter

and 200 mm in length, were filled with blood sealed at both ends with plasticine, and left at room temperature for 10 minutes. Then 5 mm of the capillaries was broken off every 30 seconds. The end point of the clotting time was taken when the blood could be drawn out as thread.

LIPID ANALYSES

Cholesterol analysis was made directly on citrated plasma by the method of Zlatkis, Zak, and Boyle (38)

Extraction procedure A portion of 5 ml of the citrated plasma was blown down into 100 ml of mixture of 2 parts of chloroform A. R. and one part of methanol A. R. at room temperature. After standing 1 hour at room temperature, the chloroform-methanol extract was filtered through glass filter number 4 and evaporated to dryness under reduced pressure and t temperature of + 50° C. The residue was reextracted with 4 successive portions of chloroform of + 50° C. The chloroform extract was filtered through fat-free filter paper into measuring flask and adjusted to 25 ml at a temperature of +20° C. On this chloroform extract the total lipid and lipid phosphorus determinations were performed. All analyses were performed in duplicate.

Lipid phosphorus was determined by the method of Fiske and Subbarow (36) as modified by Bentte (37)

Total lipids Two 5 ml portions of the chloroform extract were evaporated and the residue was weighed (37)

The figures for the lipid concentrations express the content in citrated plasma thus the figures have not been adjusted for dilution with the citrate solution.

STATISTICAL METHODS

1 The mean differences between the fasting values and the values after 1—7 hours respectively in the cream and olive oil groups were compared with the corresponding mean differences in the control group on the hypothesis of equality in changes in clotting times and lipid concentrations. Differences between two mean values are thus analyzed with Student t-test.

$$t = \frac{\bar{Y} - \bar{X}}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

Where $\bar{Y}$ is the mean difference in the cream or olive oil group, $\bar{X}_2$ the mean difference in the control group, and S_1^2 and S_2^2 the corresponding variances, and n_1 and n_2 the corresponding numbers. Tables of the probability function of t then gives the probability of the observed difference.

2. The mean differences between the fasting values and the values after 1—7 hours on a percentage basis, in the cream and olive oil groups were compared with the corresponding percentage differences in the control group. The calculation was performed as above. Here $\bar{Y}_1$ is the mean of the differences on a percentage basis in the cream or olive groups and $\bar{X}_1$ corresponding mean differences in the control group.

3. The correlation analyses were performed by ordinary statistical methods. Correlation coefficient = r

Significance levels

0.05	$\geq P > 0.01$	Significant
0.01	$\geq P > 0.001$	Highly significant
0.001	$\geq P >$	Very highly significant

Material

Healthy young students or patients with neuritis of sciatic nerve or peptic ulcer were used for the experiments. All subjects had been fasting for 13 hours before the experiment was started. Three groups were investigated, one group of 14 subjects who were given water only (control group) one group of 12 subjects who were given 3 ml of 40 cow's cream per kg body weight, and one group of 10 subjects who were given 1 ml of olive oil per kg body weight.

Results

The figures for whole blood clotting time, Russell's viper venom clotting time lipid phosphorus, and total lipids are presented in table I. In table II and III the significance levels of the differences between the control group and the olive oil and cream groups are shown, calculated both with the actual figures and on a percentage basis

Table I Whole-blood clotting time Russell's viper venom time Lipid phosphorus, and total lipids after ingestion of cream or olive oil

Control group

Hours	0	1	2	3	4	5	6	7
W. B. C. T	1,288	1 191	1,296	1,252	1,319	1,239	1,161	—
R. V. V	16.00	15 75	17 00	17.50	15.50	17 00	16.50	15.75
Lip-P	6.43	6.31	6.97	6.81	6.82	6.64	7.08	6.65
T lip.	760	865	810	745	790	840	775	635
W. B. C. T	874	855	828	945	883	712	817	874
R. V. V	15 75	16.00	16.50	17.25	15.50	16.00	16.50	16.50
Lip-P	8.60	8.60	7.85	8.08	7.98	8.15	8.48	8.35
T-lip.	720	750	735	660	705	680	730	770
W. B. C. T	1 105	1 108	1 170	1 092	1 139	1 046	1 098	958
R. V. V	17.50	17.50	16.75	18.25	18.75	18.50	19.2[illegible]	17 75
Lip-P	7 05	6.20	6.25	6.60	6.85	6.60	7.05	7.20
T lip.	770	670	650	690	710	700	735	800
W. B. C. T	1 035	1 088	1 147	1,061	998	1 066	934	1 096
R. V. V	17.50	16.50	19 00	17.50	17.50	18.00	16.75	18.50
Lip-P	6.83	7.85	7 78	7 77	7 14	7.63	7.93	—
T lip.	755	805	800	825	805	750	[illegible]90	685
W. B. C. P	1 425	1 485	1 403	1 474	1 487	1 123	1,305	1,375
R. V. V	19.00	23.75	21.00	20.00	22.[illegible]5	24.00	21.50	21.50
Lip-P	4 60	4.30	4 14	4.38	4.30	4.50	4.66	4 71
T lip.	476	456	490	440	463	490	501	535
W. B. C. T	1 191	1,283	1,214	1,301	1,281	1,208	1,314	1,036
R. V. V	[illegible]5.50	24.25	22.75	—	25.25	24.25	23 00	23.00
Lip-P	5.35	5.25	5.08	—	5.74	5.53	5.53	5.37
T lip.	600	585	570	—	565	500	600	535
W. B. C. T	1,531	1 535	1,356	1,304	1,282	1,535	1,311	1 045
R. V. V	20.00	22.75	1.00	19.00	19.25	19.[illegible]5	19 00	18.00
Lip-P	3.58	3 49	3.58	—	3 43	3.58	3.58	3.55
T-lip.	790	725	740	605	680	710	800	730
W. B. C. T	1,521	1 149	1 084	1 143	—	1 006	1 116	1 127
R. V. V	20.25	20.50	20.75	22.00	20.50	16 75	19 75	17 00
Lip-P	7.53	7 12	7 17	6.93	6.81	6.46	6.46	6.34
T-lip.								
W. B. C. T	916	983	1 002	[illegible]98	847	786	1 179	806
R. V. V	23.50	24 00	23.75	24.50	[illegible]3.50	23.50	23 50	26.00
Lip-P	5.55	6.31	5 80	6.00	6 13	6.06	6.25	6.37
T lip.	585	545	515	495	495	510	540	5[illegible]0
W. B. C. T	1,003	984	805	904	842	[illegible]86	866	806
R. V. V	22.00	23.75	22.50	[illegible]2.50	22.25	22.25	22.00	22.75
Lip-P	5.16	4.20	5.21	5 47	5.35	5 54	5 66	5.56
T-lip.	480	460	525	535	495	520	550	525
W. B. C. T	883	859	957	1 026	911	983	973	8[illegible]9
R. V. V	20.25	21.50	20.25	19 50	18 50	19 00	19.00	18.50
Lip-P	7.93	7.55	7 43	7 74	7 74	7 80	7.94	8 00
T-lip.	690	635	625	670	670	665	693	50

Table I Cont

Control group

Hours	0	1	2	3	4	5	6	7
W.B.C.T.	1,031	1,010	881	826	816	848	847	956
R.V.V.	19.50	19.50	18.00	18.00	18.50	18.50	18.00	19.00
Lip-P	7.04	7.16	7.56	7.27	7.11	7.59	7.29	7.61
T-lip.	760	760	805	740	730	765	750	790
W.B.C.T.	1,497	1,457	1,530	1,529	1,417	1,458	1,337	1,352
R.V.V.	17.50	18.00	18.00	18.25	18.50	18.50	16.50	17.00
Lip-P	7.48	7.26	7.52	7.59	7.84	7.79	7.25	7.58
T-lip.	945	955	975	1,000	990	990	980	1,030
W.B.C.T.	1,348	—	1,313	961	873	1,019	1,367	1,320
R.V.V.	16.25	16.50	15.00	16.50	16.00	16.00	16.50	16.75
Pip-P	5.47	5.05	5.28	4.75	5.71	5.59	5.46	5.78
T-lip.	660	625	660	645	665	680	690	735

Olive oil group

W.B.C.T.	938	656	666	—	703	741	733	840
R.V.V.	18.00	18.00	17.50	15.00	14.50	15.00	—	17.50
Lip-P	5.83	7.07	7.67	6.09	6.62	7.73	—	6.69
T-lip.	540	595	760	710	720	765	—	718
W.B.C.T.	1,118	1,027	956	958	974	864	735	946
R.V.V.	—	14.75	14.50	11.75	11.75	12.25	11.00	14.23
Lip-P	—	7.45	8.71	8.41	8.96	9.68	9.02	9.08
T-lip.	—	620	765	800	835	975	970	880
W.B.C.T.	1,237	1,407	1,301	1,190	991	1,001	1,008	1,081
R.V.V.	15.75	16.00	13.75	11.75	16.00	17.00	16.00	16.25
Lip-P	6.04	6.82	7.02	7.12	7.23	7.23	6.88	7.40
T-lip.	630	680	725	775	720	755	745	750
W.B.C.T.	1,135	977	951	—	860	757	741	769
R.V.V.	15.50	15.00	13.75	11.50	11.00	11.25	11.00	13.25
Lip-P	7.92	8.61	9.06	8.19	9.25	9.44	8.50	9.58
T-lip.	790	840	905	1,000	1,160	1,045	1,040	885
W.B.C.T.	873	847	846	894	815	768	829	896
R.V.V.	17.50	17.75	17.75	19.25	18.00	17.50	17.75	17.50
Lip-P	8.07	7.91	8.03	7.53	7.73	7.84	8.05	7.98
T-lip.	730	810	815	715	715	720	740	740
W.B.C.T.	958	952	937	759	773	659	795	890
R.V.V.	13.50	15.25	13.50	11.50	9.50	10.75	11.25	13.25
Lip-P	6.11	6.90	7.01	7.13	7.50	7.24	7.13	7.19
T-lip.	600	640	680	695	780	760	715	595
W.B.C.T.	856	848	781	—	765	768	843	804
R.V.V.	17.25	16.50	14.00	12.23	9.50	8.50	9.23	13.50
Lip-P	7.50	7.22	7.60	7.94	8.72	9.01	—	8.67
T-lip.	830	780	840	890	1,070	1,103	—	940

Table I Cont

Olive oil group

Hours	0	1	2	3	4	5	6	7
W B. C. T	1 075	1.045	890	870	963	906	838	981
R. V V	18.75	17.50	18.25	17.25	16.50	15.00	15.00	19.50
Lip-P	7.30	6.38	7.00	7.30	7.20	7 78	7.24	7 41
T lip.	700	720	710	760	750	780	765	750
W B. C. T	1 023	984	1,016	1,016	1,021	925	812	833
R. V V	15.75	15 75	15.50	13 00	13.00	13.50	13.00	12.50
Lip-P	7.52	7.90	7.90	6.65	7.88	8.02	7.96	8.09
T lip.	695	730	770	850	845	825	875	845
W B. C. T	1 177	986	947	965	763	849	937	969
R. V V	17 00	14 75	13.50	11.00	10.00	10.25	11.00	13.75
Lip-P	7.97	7.91	8.20	8.38	8.72	8.66	8.78	8.49
T lip.	795	865	960	1 160	1,325	1,215	1 150	1 050

Cream group

W B. C. T	1,314	954	1,207	1,241	912	803	900	902
R. V V	20.50	24.00	21 00	19.00	17.25	14.50	18.00	22.50
Lip-P	6.00	6.00	6.87	6.62	6.62	7.25	7.25	7.25
T lip	650	615	695	730	720	635	665	623
W B. C. T	1,314	724	712	789	684	1 012	1,282	—
R. V V	30.00	24 00	24.00	24.50	19 75	21 00	24.00	—
Lip-P	4.96	—	5 60	5 44	5 45	5.36	6.17	—
T lip.	515	555	780	625	515	565	580	—
W B. C. T	1,500	1 404	1 435	925	927	977	1,368	1,500
R. V V	26.50	27 00	24 00	24.50	20 00	21.00	27.00	27.50
Lip-P	3.82	4 19	3.96	3.99	4.59	4 49	4 70	4.27
T-lip.	675	545	570	690	565	575	680	530
W B. C. T	1 149	874	1,002	1 118	860	836	748	788
R. V V	29.50	28.50	16.00	17.00	17.00	15.00	16.00	17.50
Lip-P	6 70	5.82	6.23	6.84	7.62	6.83	7.01	7.37
T-lip.	800	705	785	910	910	770	925	1,535
W B. C. T	1,247	1 128	1 106	1 147	1 122	913	875	958
R. V V	23.00	24.00	20 75	20 75	21.50	18.00	18.00	—
Lip-P	2.74	2.68	3 46	3.93	4 41	2.44	3.70	4 17
T-lip.	490	485	550	600	650	550	575	600
W B. C. T	1,333	1,358	1,671	1,374	1,787	804	1 784	1,698
R. V V	19 00	19.50	19 00	18 75	19.50	20 00	19.50	18.75
Lip-P	4 77	4 71	4 05	5 01	6.68	5 42	7 04	6.84
T lip	590	580	665	675	665	710	630	603
W B. C. T	1,920	1 166	1 127	1,022	925	838	1 169	1,094
R. V V	20.00	18.25	15.75	12.50	12.25	17 00	18.50	17 00
Lip-P	7.87	7.63	7.95	8.27	8.59	8 23	9.07	8.54
T lip.	720	780	790	840	890	800	830	785

Table I. Cont.

Cream group

Hours	0	1	2	3	4	5	6	7
W. B. C. T	1,535	1,018	946	1,022	833	758	890	989
R. V. V.	27.50	23.50	24 25	23.00	21.00	21.50	22.25	23.50
Lip-P	4.38	4.32	4.14	4 87	4.38	4.56	4.87	4.93
T-lip.	435	485	525	580	570	550	475	420
W. B. C. T	933	1,224	856	1,209	700	735	1,097	1 188
R. V. V.	15.50	14.25	11.50	13 00	15.00	14.25	14.25	13.25
Lip-P	8.10	8.13	8.45	8.78	9.00	9.93	9.18	7 40
T-lip.	920	970	990	1,050	1,005	1,025	1,030	1,005
W. B. C. T	996	862	821	855	634	840	1,023	952
R. V. V.	14 75	15 00	12.25	12.00	13.75	14 00	13.50	16.75
Lip-P	7 77	7.88	8.07	8.12	7.88	8 23	8.18	8.12
T-lip.	700	740	800	805	750	760	760	685
W. B. C. T	1 415	1,545	1,371	1,120	855	904	1,061	1,207
R. V. V.	18 50	16.25	14.75	11.75	12.75	12.75	15.00	16.25
Lip-P	5.22	5 33	5.56	6 07	6.20	6.50	6.05	5.98
T-lip	660	745	890	915	930	850	890	770
W. B. C. T	1,394	1 409	1,631	1,215	1,027	545	1 104	915
R. V. V.	26.00	27 50	27.50	17.00	19 00	19.50	21.50	28 00
Lip-P	6.40	6.78	6.50	6.80	7.00	7.00	7.20	6 70
T-lip.	615	650	980	750	800	720	885	670

W. B. C. T – whole-blood clotting time in seconds.
R. V. V. – Russell viper venom time in seconds.
Lip-P – lipid phosphorus in mg 100 ml.
T-lip. – total lipids in mg 100 ml.

Table II Significance levels of the differences between the cream group and the control group

	Hours						
	1	2	3	4	5	6	7
W B C T actual figures				× ×	×		
W B C T percentage-wise				× ×	× × ×		
R V V actual figures			× ×	× × ×	×		
R V V percentage-wise			× × ×	× × ×			
Lip-P actual figures				× ×			
Lip-P percentage-wise		×	×	× ×			
T-lip. actual figures			×	×	×		
T-lip. percentage-wise		× ×	× × ×	×			

Prothrombin-proconvertin (PP) prothrombin, proaccelerin, cholesterol, platelets, red cells and haemoglobin concentration did not vary significantly in any of the groups.

Table III Significance levels of the differences between the olive oil group and the control group

	Hours						
	1	2	3	4	5	6	7
W B. C. T actual figures							
W B. C. T percentage wise						× ×	
R. V V actual figures			× ×	× ×	×	× ×	
R. V V percentage-wise			× × ×	× ×	× ×	× ×	
Lip-P actual figures				×	×		×
Lip-P percentage wise		× ×		×	× ×		×
T-lip. actual figures			×	× ×	× ×	× ×	
T-lip. percentage wise		× ×	× × ×	× × ×	× × ×	× × ×	×

Correlations between the clotting tests and between these and the lipid concentrations after ingestion of cream

The analyses presented below were made to investigate if there was any correlation between W B C. T and R. V V and between each of these clotting tests and the total lipid or lipid phosphorus concentrations. The calculation was made on the values in the cream group 3 4 and 5 hours after the meal (i. e. n = 3 × 12 = 42) Log of the clotting times was also included This has been done because there is a possibility that the log of the clotting times represents the degree of hypercoagulability of the blood more truly than the real figures There is for instance a linear proportionality between log clotting time and log thrombin concentration in isolated systems

The following correlations were calculated

1) W B C T and R. V V both actual figures (r = 0 18)

2) W B C. T and R V V both percentage wise (r = 0 29)

3) Log W B. C. T and log R V V (r = 0.27)

4) W B C. T and T lip both actual figures (r = 0 002)

5) W B C. T and T lip both percentage wise (r = 0 05)

6) Log W B C. T and T lip actual figures (r = 0 025)

7) W B C. T and Lip-P both actual figures (r = 0 09)

8) W B C. T and Lip-P both percentage-wise (r = 0.2)

9) Log W B C T and Lip P actual figures (r = 0 07)

10) R. V V and T Lip both actual figures (r = 0 04)

11) R. V V and T lip both percentage wise (r = 0 12)

12) Log R V V and T lip. actual figures (r = 0 12)

13) R V V and Lip P both actual figures (r = 0 31)

14) R V V and Lip P both percentage wise (r = 0 32)

15) Log R V V and Lip P actual figures (r = 0 31)

None of these calculations revealed any significant correlations. The same calculations as above were also done separate for each one of the three groups of values (i. e. after 3 4 and 5 hours, thus n = 12) In this calculation a significant correlation between W B C. T and R V V (r = 0 86 P > 0 001 ++) both on a percentage basis was found after 4 hours but not

after 3 and 5 hours. Furthermore R. V V and Lip-P both on a percentage basis seemed to be correlated (r = 0.56) after three hours.

Correlations between the clotting tests and the lipid concentrations in the fasting state

It has been reported that patients with hyperlipemia have shorter R. V V in the fasting state than normal persons (9 19) The W B. C. T of atherosclerotic patients, furthermore, has been reported to become longer when the lipid level in the blood diminishes during therapy with unsaturated fats (20) To investigate if there was any correlation between the clotting times and the lipid levels in the fasting state in this normal material, the following correlations were calculated on all subjects in the three groups (n = 36)

1) Fasting W B. C. T and T lip., both actual figures (r = 0.07)

2) Log fasting W B. C. T and T lip actual figures (r = 0.10)

3) Fasting W B. C. T and Lip-P both actual figures (= 0.41 $0.05 \geq P >$ 0.01)

4) Log fasting W B C. T and Lip-P actual figures (r = 0 46 $0 01 \geq P >$ 0.001)

5) R. V V and T-lip., both actual figures (r = 0.43 $0.01 \geq P > 0 001$)

6) Log R V V and T-lip., actual figures (r = 0.44 $0 001 \geq P > 0.001$)

7) R. V V and Lip-P both actual figures (= 0.51 $0 01 \geq P > 0.001$)

8) Log R. V V and Lip-P actual figures (= 0.5., $0 001 \geq P$)

In this normal material there are significant correlations between the clotting times and the lipid levels in the fasting stat

Correlations between the length of the fasting W B C. T and the degree of shortening after cream ingestion

The shortening of the W B C. T after a fatty meal is said to be more pronounced when the fasting W B C. T is long (41) The correlation was, therefore, calculated between the fasting values of the W B C. T in the cream group and the maximal shortenings on a percentage basis (r = 0.69 $0.05 \geq P > 0 01$*) This is probably an important point which might explain some of the differing results obtained by the many investigations on the influence of a fatty meal on the coagulation of blood.

Discussion

On the basis of this investigation it can be concluded that after a meal of 3 ml of cream per kg body weight, there is a significant shortening of the whole blood clotting time and Russell's viper venom time, and after a meal of 1 ml of olive oil per kg body weight there is a significant shortening of Russell's viper venom time. There is a significant increase of both the total lipid and the lipid phosphorus concentrations, but there is no significant correlation between the increased lipid concentrations and the changes of the clotting times. This lack of correlation does not mean that the fat is not responsible for the increased clottability of the blood When the balance between procoagulants and anticoagulants in vivo is disturbed a chain of reactions probably is tarted and what we are able to observe at a particular time will be the resultant of activation and inactivation reactions.

There is no significant correlation between the whole blood clotting time and Russell s viper venom time, except for the single values after 4 hours. The whole

blood clotting time reflects the whole intrinsic blood coagulation system Russell's viper venom time only a limited part of it. It is not surprising that they do not exactly parallel each other

As yet we do not know if an increased clottability of the blood as judged by the clotting tests in vitro increases the tendency to form intravascular thrombosis. The only way to learn this would be to correlate the clotting tests with the frequency of thrombo-embolic episodes in the same way as we try to get knowledge of the benefit of anticoagulant therapy

There are however certain observations which speak in favour of the theory that the fat content in foods influences the frequency of thrombosis (52, 53 54)

Summary

Blood coagulation and lipid analyses were performed on three groups of normal human subjects. To one group of 12 subjects cow's cream was given and to another group of 10 subjects olive oil. A group of 14 served as controls.

After a meal of 3 ml cow's cream per kg body weight there was a significant shortening of the whole blood clotting time and Russell's viper venom time. A meal of 1 ml of olive oil per kg body weight gave a significant shortening of Russell's viper venom time but not of the whole blood clotting time.

In both groups, there was a significant increase of total lipids and lipid phosphorus, but there was no correlation between the decrease of the clotting times and the increase of the lipid concentrations

There were significant correlations between clotting tests and lipid concentrations in the fasting state calculated on all three groups of subjects.

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XXVIII *Nordiska kongressen för invärtes medicin* hålles i Lund-Malmö den 13—16 juni 1962. President: Prof. Haqvin Malmros, Lund.

Huvudämnen. Akut njurinsufficiens, renala orsaker till hypertoni, toxiska njurskador främst fenacetinproblemet, pyelonephrit samt de medfödda metaboliska rubbningarna.

Anmälan om föredrag skall vara generalsekreteraren tillhanda före den 1 mars 1962 och kortfattade referat av föredragen före den 1 april 1962 Anmälan om deltagande senast den 15 april 1962.

Generalsekr.. Doc. Åke Nordén, Lasarettet, Lund.

Det 21 Nordiska Lungläkarmötet äger rum i Lund den 18—19 juni 1962

International congress on hormonal steroids will be held in Milan Italy in May 1962 Honorary president. Dr Gregory Pincus. General chairman Prof. Emilio Trabucchi.

The scientific programme includes a) Six symposia at which lectures will be given by prominent invited speakers; b) Sessions of short communications; c) Private scientific meetings and round table discussions.

Official languages will be English, French and Italian. Simultaneous translation.

Information, application forms and forms for submission of papers will be obtained from the secretaries. Prof. P Martini and Prof. A. Pecile, Istituto di Farmacologia e Terapia, 21 Via A. del Sarto Milan, Italy

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From disability to work." Cambridge study course July 1st—7th 1962

The British Council for Rehabilitation of the Disabled, under the patronage of H. R. H. the Duke of Edinburgh, announce a European International Seminar to be held at Cambridge University in the Summer of 1962 During this Seminar papers will be read by over thirty leading specialists representing eleven different countries.

A fee of £10 sterling will be charged for attending the course — cheques should be made out to "British Council for Rehabilitation of the Disabled (Seminar) — Residential accommodation can be arranged in advance through the British Council for Rehabilitation of the Disabled. — All enquiries should be addressed to: The Conference Secretary British Council for Rehabilitation of the Disabled Tavistock House (South) Tavistock Square London W C 1

York Association, Inc. New York 1961 542 pp

Survey Report of the Cerebral Vascular Study Group Institute of Neurological Diseases and Blindness National Institutes of Health Bethesda, Md. U S A. 1961

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The Nature of Essential Hypertension. By Sir George Pickering 149 pp Price 22s 6d J & A Churchill Ltd London 1961

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Inhaled Particles and Vapours Proceedings of an International Symposium organized by the British Occupational Hygien Society Oxford 29 March 1 April 1960 Edited by C. N Davies 493 pp. Price £5 Pergamon Press Ltd Oxford 1961

The Physiological Regulation of Salivary Secretions in Man. A study of the Response of Human Salivary Glands to Reflex Stimulation By Alexander C. Kerr 86 pp Price 40s. net. Pergamon Press Oxford-London-New York Paris 1961

Therapie-Fibel der inneren Medizin. Für Klinik und Praxis 662 S 107 Abb. Preis DM 38 Georg Thieme Verlag, Stuttgart 1961

Contribution à la biochimie des obésités expérimentales Par J Christophe. 220 p. Editions Arscia S A. Bruxelles 1961

Zur Problematik der Multiplen Sklerose Von F Georgi P Hall und H. R Müller 123 S S Karger Basel-New York 1961

Pepsinogen in Blood and Urine By M Borch Jorgensen 108 pp. Price d Kr 15. Munksgaard, Copenhagen 1961

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Psychologie des accidents Par P Aboulker L. Chertok et M Sapir 172 p Prix 9 50 NF Expansion Scientifique Française, Paris 1961

Les ostéo-arthropathies nerveuses Par A. M. Recordier P Mouren et G Serratrice. 172 p. 50 fig Prix 28 NF Expansion Scientifique Française, Paris 1961

Cerebral Infarction. The Role of Stenosis of the Extracranial Cerebral Arteries By Peter O Yates and Edward C. Hutchinson. 95 pp Price 14s. net Her Majesty's Stationery Office London 1961

Storungen des Gasaustausches in der Lunge Von Max Scherrer 136 S 23 Abb. 26 Tab Preis Fr DM 26 80 Medizinischer Verlag Hans Huber Bern und Stuttgart 1961

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L elettroencefalogramma in medicina del lavoro Di Carlo Serra e Luigi Ambrosio. 313 p Acta Neurologica, Napoli 1961

Acta Medica Scandinavica. Vol. 171, fasc. 2, 1962

From Medical Department E, Frederiksberg Hospital (Head. N I Nissen M. D)
Copenhagen, Denmark

Further Study of the Mechanism of Reduction of Erythrocyte Survival Time in Phenacetin Habitués and its Relation to "Phenacetin Kidney Disorders"

By

N I Nissen and Th. Faris

Many investigators have demonstrated that phenacetin may cause a reduction in the life-span of erythrocytes (5 6 9 18, 20) and shortened survival in haemolytic anaemia (3 5 6 18, 19 20 21 22, 23) In a previous study (5, 26) the writers expressed the opinion that it was particularly in phenacetin habitués with severe renal insufficiency that reduction of the erythrocyte survival time could be demonstrated during phenacetin ingestion, determined by means of the chromium technique (7 16) On the other hand, patients with severe renal insufficiency who had not taken phenacetin and to whom no phenacetin was given during the determination, had a practically normal erythrocyte survival time.

A subsequent work reported that it was probably plasma factor that influenced the erythrocytes, and that prednisone could minimize the reduction in erythrocyte survival time caused by phenacetin (6) This would indicate a sensitizing mechanism, though it probably could not be excluded that phenacetin or its breakdown products might affect the erythrocytes directly Lorentzen et al. (18 19) McGibbon et al. (21) and Muirhead et al. (24) also report that it may be a question of sensitization.

It is thus not yet elucidated to what extent it is merely an acquired sensitization to phenacetin or its breakdown products that causes haemolysis, or whether these cause direct toxic damage, probably linked with the fact that genetically the erythrocytes may be less resistant to drugs (see Dern et al. (4) as regards primaquin and Houston & Barlow (13) as regards phenacetin)

The aspect of a possible relationship between impairment of the kidneys and the haemolytic anaemia caused by phenacetin is also as yet undetermined.

The present study covers examination of a new control series of patients with renal insufficiency who were not addicts

Submitted for publication July 7 1961

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Table II Erythrocyte survival time and renal function in phenacetin addicts with renal failure on administration of phenacetin

Pt. No	Sex	Age	Hgb. >80 %	Sulph-hgb. ≥2 %	Reticulocytes <10 ‰	Haptoglobin 35—170 mg%	Renal function			Erythrocyte survival time T/2 days 24—36 days	
							Sp. gr 10³ phm	Creatinine			
								Clearance >67 ml/min.	mg% in serum <1.4 mg %		
1	F	54	52	10.0—4.6	34	—	08—13	3	10.45	9	
			53	0	7	300		2.50	12.58		*23*
2	F	52	44	9.0	50	10	07—10	7	7.73	11	
			52	3.5	10	113			9.83		*44*
3	M	60	48	7.1—4.6	13	—	09—15	8	7.40	14	
4	F	78	71	3.8	63	—	10—14	16	2.43	14	
			81	0	6	112		—	2.48		*47*
5	F	48	50	11.1—1.3	11	—	09—13	2	14.70	15	
6	M	55	69	2.4	31	124	09—17	—	3.48	15	
7	M	75	65	9.6	10	—	07—16	3.9	7.57	16	
			79	0	8	89		4.7	9.35		*23*
8	F	60	80	0	17	370	11—18	31.0	2.21	16	
9	F	60	57	8.7	5	—	08—11	24.0	2.84	17	
			96	0.3	6	246		—	2.09		*40*
10	F	53	145	6.6—3.9	15	—	06—09	—	6.72	18	
11	F	56	63	4.2	3	32	09—14	21.0	2.31	18	
12	F	43	62	1.0	13	7	08—12	24.0	3.31	19	
13	M	56	90	0	5	178	12—14	28.0	3.17	19	
14	M	63	60	1.5	29	—	13—16	35.0	1.59	20	
15	F	55	72	5.8	11	—	15—18	18.0	3.81	22	
16	F	63	74	1.5	6	408	09—15	13.0	2.19	24	
17	F	54	103	—	4	—	13—18	31.0	1.86	25	
18	F	76	70	2.9	6	193	15—20	16.0?	1.63	26	
19	F	64	57	3.2	13	—	06—09	9.0	5.78	28	
20	M	77	68	2.8	7	—	19—21	46.0	1.83	30	
21	M	45	71	2.3	10	102	03—05	37.0	1.89	33	
22	F	62	86	0.5	7	100	10—17	33.0	1.82	35	
		Mean	66.3	4.09	16.6	132.3	10—14	20.3	6.70	20.2	

Type 0 blood corpuscles used for determination of erythrocyte survival time, since blood transfusion had been performed previously

The erythrocyte survival time was also determined for these patients *without* administration of phenacetin during the experiment.

Four patients (Nos. 1, 6, 7 and 10) had papillary necrosis.

Group II 22 phenacetin habitués *with* renal insufficiency *with* ingestion of phenacetin during the test (1.5 g daily)

Group II a. 5 of the group II patients *without* ingestion of phenacetin during the test.

Table I Erythrocyte survival time and renal function in phenacetin addicts without renal insufficiency on administration of phenacetin

Pt. No.	Sex	Age	Hgb. >80 %	Sulph hgb. ≦2 %	Reticulocytes <10 /‰	Haptoglobin 35—170 mg%	Renal function			Erythrocyte survival time $\frac{T}{2}$ days 24—36 days
							Sp gr 10³ plus	Creatinine		
								Clearance >67 ml/min.	mg** in serum <1 4 mg %	
1	F	45	84	3 4—0.7	4		13—18	99	0.90	20
2	F	51	86	0.2	2	266	16—20	74	1.08	21
3	F	45	72	4 7—0.6	6	—	15—23	52	0.90	23
4	F	61	75	10.0—5.3	6	—	18—25	68	0.92	23
5	F	74	62	5.8—2.3	12	—	16—19	42	1.23	23
6	F	42	83	3 7	12	—	09—15	56	1.32	23
7	F	41	91	0	4	86	12—22	67	0 89	23
8	M	63	83	2 1	4	—	18—25	86	1.30	26
9	M	48	62	5.2—3 0	10	—	15—22	122	1 18	29
10	M	73	52	2.8	3	—	20—21	—	1.39	29
11	F	42	71	4.6	10	—	21—26	59	1.05	31
12	F	56	86	1 0	4	84	10—17	52	1.26	34
13	F	63	67	2.6	6	—	17—18	50	1.25	35
14	F	75	102	2 1	14	—	18—25	51	1.36	37
15	M	65	78	4.8	3	—	13—18	60	1 40	37
16	F	29	83	2.0—3.2	6	—	uncertain	70	1.00	38
		Mean	77.3	3 18	6.9		15—21	67.2	1 15	28 3

The first figure is the average of all the daily specific gravity values during admission, and the second figure the average of the three highest values observed.
Later a serum creatinine valve of 2.23—1 60 mg%
Admitted to hospital three weeks later with uraemic pyelonephritis and subsequently admitted three times.
During hospitalization discharge of the kidney papilla verified by microscopy Four months later admitted with uraemic pyelonephritis (No. 16 in table II) Subsequently admitted three times.

to phenacetin. Here determination of erythrocyte survival time was performed both *with* and *without* simultaneous ingestion of phenacetin this being necessary in order to evaluate the haemolytic anaemia in the phenacetin addicts. It also gives information concerning some broader determinations of erythrocyte survival time in phenacetin addicts, and includes discussion of the results in relation to kidney disorders.

The term "phenacetin habitués covers patients with a definite daily abuse of phenacetin who up to the time of admission had taken at last 1 5 g phenacetin daily for one or several years. Patients who were previously habitués but who had not taken the drug for at least six months were not included in the category

Determination of erythrocyte survival time was carried out on the following patients

Group I 16 phenacetin habitués *without* renal insufficiency i. e. with normal serum creatinine *with* ingestion of phenacetin during the test (1.5 g daily)

Table II. Erythrocyte survival time and renal function in phenacetin addicts with renal failure on administration of phenacetin

Pt. No	Sex	Age	Hgb. >80 %	Sulph-hgb. ≥2 %	Reticulocytes <10 ‰	Hapto-globin 35—170 mg%	Renal function			Erythrocyte survival time $\frac{T}{2}$ days 24—36 days	
							Sp. gr 10³ plus	Creatinine: Clearance >67 ml/min.	Creatinine: mg% in serum <1 4 mg %		
1	F	54	52	10.0—4 6	34	—	08—13	3	10.45	9	
			55	0	7	500		2.50	12.38		*23*
2	F	52	44	9 0	50	10	07—10	7	7.73	11	
			52	3.5	10	113			9 83		*44*
3	M	60	48	7.1—4.6	13	—	09—15	8	7 40	14	
4	F	78	71	3.8	65	—	10—14	16	2.43	14	
			81	0	6	112		—	2.48		*47*
5	F	48	50	11 1—1.3	11	—	09—13	2	14.70	15	
6	M	55	69	2.4	31	124	09—17	—	3 48	15	
7	M	75	63	9.6	10		07—16	3.9	7.37	16	
			79	0	8	89		4.7	9.35		*23*
8	F	60	80	0	17	370	11—18	31.0	2.21	16	
9	F	60	57	8 7	5	—	08—11	24 0	2.84	17	
			96	0 3	6	246		—	2.09		*40*
10	F	53	143	6.6—3.9	15	—	06—09	—	6 72	18	
11	F	56	63	4.2	3	32	09—14	21.0	2 31	18	
12	F	45	62	1 0	13	7	08—12	24 0	3.31	19	
13	M	56	90	0	5	178	12—14	28.0	3 17	19	
14	M	63	60	1.5	29	—	13—16	35.0	1.39	20	
15	F	55	72	5.8	11	—	15—18	18 0	3 81	22	
16	F	65	74	1.5	6	408	09—15	13.0	2.19	24	
17	F	54	103	—	4	—	13—18	31.0	1.86	25	
18	F	76	70	2 9	6	193	15—20	16 07	1 65	26	
19	F	61	57	3 2	13	—	06—09	9.0	5.78	28	
20	M	77	68	2.8	7	—	19—21	46 0	1.83	30	
21	M	45	71	2 3	10	102	03—05	37 0	1 09	33	
22	F	62	86	0.5	7	100	10—17	33 0	1.82	35	
		Mean	66 3	4 09	16 6	152 3	10—14	20.3	6 70	20.2	

Type 0 blood corpuscles used for determination of erythrocyte survival time, since blood transfusion had been performed previously

The erythrocyte survival time was also determined for those patients *without* administration of phenacetin during the experiment

Four patients (Nos. 1 6, 7 and 10) had papillary necrosis.

Group II 22 phenacetin habitués *with* renal insufficiency with ingestion of phenacetin during the test (1.5 g daily)

Group II a. 5 of the group II patients *without* ingestion of phenacetin during the test.

Table I. Erythrocyte survival time and renal function in phenacetin addicts without renal insufficiency at administration of phenacetin

Pt. No.	Sex	Age	Hgb. >80 %	Sulph hgb ≦2 %	Reticulocytes <10 /₀₀	Haptoglobin 35—170 mg%	Renal function: Sp gr 10* plus	Renal function: Creatinine: Clearance >67 ml/min.	Renal function: Creatinine: mg% in serum <1.4 mg %	Erythrocyte survival time $\frac{T}{2}$ days 24—36 days
1	F	45	84	3.4—0.7	4		13—18	99	0.90	20
2	F	51	86	0.2	2	266	16—20	74	1.08	21
3	F	45	72	4.7—0.6	6	—	15—23	52	0.90	23
4	F	61	73	10.0—5.3	6	—	18—25	68	0.92	23
5	F	74	62	5.8—2.3	12	—	16—19	42	1.23	23
6	F	42	83	3.7	12	—	09—15	56	1.32	23
7	F	41	91	0	4	86	12—22	67	0.89	23
8	M	63	83	2.1	4	—	18—25	86	1.30	26
9	M	48	62	5.2—3.0	10	—	15—22	122	1.18	29
10	M	73	52	2.8	3	—	20—21	—	1.39	29
11	F	42	71	4.6	10	—	21—26	59	1.05	31
12	F	56	86	1.0	4	84	10—17	52	1.26	34
13	F	63	67	2.6	6	—	17—18	50	1.25	35
14	F	75	102	2.1	14	—	18—25	51	1.36	37
15	M	65	78	4.8	3	—	13—18	60	1.40	37
16	F	29	83	2.0—3..	6	—	uncertain	70	1.00	38
		Mean	77.3	3.18	6.9		15—21	67.2	1.15	28.3

The first figure is the average of all the daily specific gravity values during admission, and the second figure the average of the three highest values observed.

Later a serum creatinine value of 2.23—1.60 mg%.

Admitted to hospital three weeks later with uraemic pyelonephritis and subsequently admitted three times.

During hospitalization discharge of the kidney papilla verified by microscopy. Four months later admitted with uraemic pyelonephritis (No. 16 in table II). Subsequently admitted three times.

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The term phenacetin habitués covers patients with a definite daily abuse of phenacetin who up to the time of admission had taken at last 1.5 g phenacetin daily for one or several years. Patients who were previously habitués but who had not taken the drug for at least six months were not included in the category.

Determination of erythrocyte survival time was carried out on the following patients.

Group I. 16 phenacetin habitués *without* renal insufficiency, i. e. with normal serum creatinine *with* ingestion of phenacetin during the test (1.5 g daily).

Table II. Erythrocyte survival time and renal function in phenacetin addicts with renal failure on administration of phenacetin

Pt. No	Sex	Age	Hgb. >80 %	Sulph-hgb. ≧2 %	Reticulo-cytes <10 ‰	Hapto-globin 55—170 mg%	Renal function: Sp. gr 10³ plus	Renal function: Creatinine Clearance >67 ml/min.	Renal function: Creatinine mg% in serum <1.4 mg%	Erythro-cyte sur vival time T/2 days 24—36 days	
1	F	54	52	10.0—4.6	34	—	08—13	3	10.45	9	
			53	0	7	300		2.50	12.38		*23*
2	F	52	44	9.0	50	10	07—10	7	7.73	11	
			52	3.5	10	115			9.63		*44*
3	M	60	48	7.1—4.6	13	—	09—15	8	7.40	14	
4	F	78	71	3.8	65	—	10—14	16	2.43	14	
			81	0	6	112		—	2.48		*47
5	F	48	50	11.1—1.3	11	—	09—13	2	14.70	15	
6	M	55	69	2.4	31	124	09—17	—	3.48	15	
7	M	75	65	9.6	10	—	07—16	3.9	7.57	16	
			79	0	8	89		4.7	9.33		*23*
8	F	60	80	0	17	370	11—18	31.0	2.21	16	
9	F	60	57	8.7	3	—	08—11	24.0	2.84	17	
			96	0.3	6	246		—	2.09		*40*
10	F	53	45	6.6—3.9	15	—	06—09	—	6.72	18	
11	F	56	65	4.2	3	32	09—14	21.0	2.31	18	
12	F	45	62	1.0	13	7	08—12	24.0	3.31	19	
13	M	56	90	0	5	178	12—14	28.0	3.17	19	
14	M	63	60	1.5	29	—	13—16	35.0	1.59	20	
15	F	55	72	5.8	11	—	15—18	18.0	3.81	22	
16	F	65	74	1.5	6	408	09—15	13.0	2.19	24	
17	F	54	103	—	4	—	13—18	31.0	1.86	25	
18	F	76	70	2.9	6	193	15—20	16.0?	1.65	26	
19	F	64	57	3.2	13	—	06—09	9.0	5.78	28	
20	M	77	68	2.8	7	—	19—21	46.0	1.83	30	
21	M	43	71	2.5	10	102	03—05	37.0	1.89	33	
22	F	62	86	0.5	7	100	10—17	33.0	1.82	35	
		Mean	66.3	4.09	16.6	152.3	10 14	20.5	6.70	20.2	

Type 0 blood corpuscles used for determination of erythrocyte survival time, since blood transfusion had been performed previously.

The erythrocyte survival time was also determined for these patients without administration of phenacetin during the experiment.

Four patients (Nos. 1 6, 7 and 10) had papillary necrosis.

Group II. 22 phenacetin habitués *with* renal insufficiency *with* ingestion of phenacetin during the test (1.5 g daily).

Group II a. 5 of the group II patients *without* ingestion of phenacetin during the test.

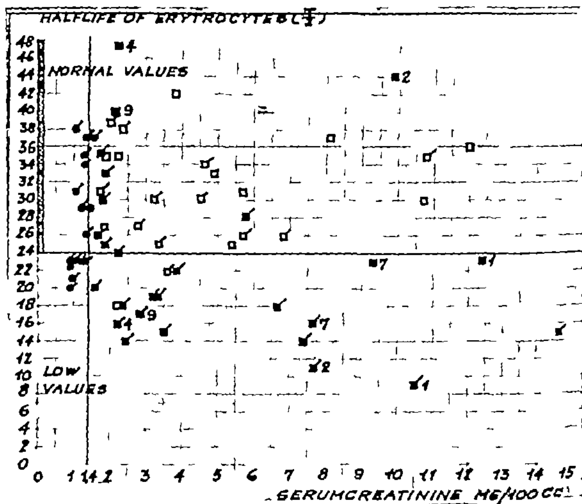


Fig 1 Erythrocyte half life (T) in relation to serum creatinine.

- ● Phenacetin addicts without renal insufficiency taking 1.5 g phenacetin daily during survival time determination (16 cases)
- ■/ Phenacetin addicts with renal insufficiency taking 1.5 g phenacetin daily during survival time determination (22 cases)
- ■ Phenacetin addicts with renal insufficiency not taking phenacetin during survival time determination (Nos. 1 2 4 7 9 in group II)
- □ Non-addicts with renal insufficiency not taking phenacetin during survival time determination (11 cases)
- □ Non-addicts with renal insufficiency taking 1.5 g phenacetin daily during survival time determination (12 cases)

Group III 11 *non*-addicts with renal insufficiency *without* ingestion of phenacetin during the test.

Group IV 12 *non* addicts *with* ingestion of phenacetin during the test (1.5 g daily)

Table I shows the patients in group I The reason for the phenacetin abuse (generally of many years duration) was headache migraine myositis, polyarthritis, osteo-arthrosis and psychopathology All the patients in group II had chronic pyelonephritis. It has not been taken into consideration that this might *primarily* have been due to causes other than the use of phenacetin One patient had been previously but was no longer

Table III. Erythrocyte survival time and renal function in non-addicts to phenacetin with renal insufficiency without administration of phenacetin

Pt. No.	Sex	Age	Hgb. >80 %	Sulph-hgb. ≦2 %	Reticulocytes <10 ‰	Haptoglobin 35—170 mg%	Renal function			Erythrocyte survival time $\frac{T}{2}$ days 24—36 days
							Sp gr 10^3 plus	Creatinine		
								Clearance >67 ml/min.	mg% in serum <1.4 mg %	
1	F	80	58	0	8	—	09—11	19	2.21	18
2	F	64	59	2.5	8	—	08—12	—	5.35	25
3	M	74	55	1.9	23	—	09—16	50	1.83	27
4	M	67	79	0.3	8	51	06—07	16.2	10.8	30
5	F	70	52	6.3?	8	—	06—09	11	5.65	31
6	F	58	59	0.5	8	—	10—17	15	4.91	33
7	F	49	71	2.8	28	—	08—14	30	1.90	35
8	F	70	64	0	11	—	10—17	31	2.25	35
9	M	81	54	3.9	8	—	09—12	—	12.0	36
10	M	60	75	0.1	8	—	07—11	8	8.10	37
11	F	57	72	0.9	3	—	05—08	19	3.84	42
		Mean	63.5	1.73	11		8—12	22.1	5.35	31.7

Type 0 blood corpuscles used for determination of erythrocyte survival times, since blood transfusion had been performed previously

No. 1 was a phenacetin addict up to 1957 and No. 2 up to 1958, but subsequently took less than 1 gram per day; No. 3 was a phenacetin addict up to 1957 and No. 4 up to 1957. No. 6 was a typical migraine subject who had abused saridon until six months previously and likewise No. 7 who had abused phenacetin until ten months previously. None had papillary necrosis.

under observation for nephrolithiasis, and one had slight diabetes mellitus. In these patients also phenacetin ingestion might have caused secondary disorders. All the patients in group III had chronic pyelonephritis, except No. 5 who had chronic glomerulonephritis, and Nos. 9 and 10 who had nephrosclerosis. In group IV all the patients had also chronic pyelonephritis, except Nos. 4 and 10 who had chronic glomerulonephritis and No. 3 who had cystic kidney. None had haematuria or other bleeding and none were given blood transfusion during the investigation though transfusion had been made previously in a few cases. These latter were therefore given labelled type 0 erythrocytes from healthy subjects.

Results

The normal value for the biological erythrocyte half life ($\frac{T}{2}$) is 24—36 days, as calculated by the method described previously (5, 7, 16). The experimental results are given in tables I—IV and figs. 1 and 2. Measurement of serum creatinine and creatinine clearance was used as an indication of the kidney function. This is probably somewhat inadequate as the earliest kidney parenchymal damage which occurs in phenacetin abuse is said to be tubular or interstitial. Reduction of creatinine clearance could therefore be expected to occur at a later date and to indicate more comprehensive renal impairment.

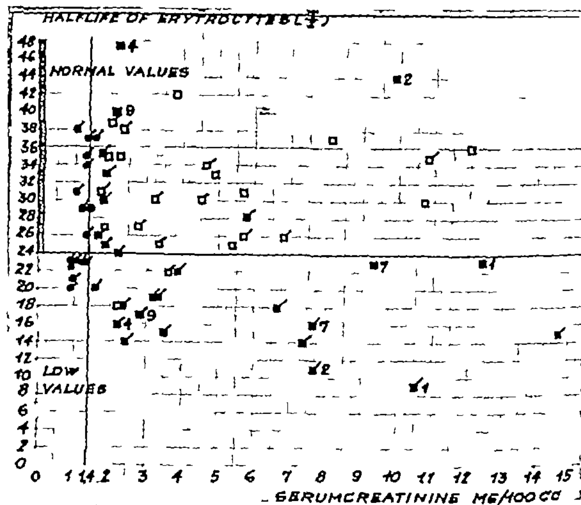


Fig. 1 Erythrocyte half-lif (T) in relation to serum creatinine.

- Phenacetin addicts without renal insufficiency taking 1 5 g phenacetin daily during survival time determination (16 cases)
- Phenacetin addicts with renal insufficiency taking 1.5 g phenacetin daily during survival time determination (22 cases)
- Phenacetin addicts with renal insufficiency not taking phenacetin during survival time determination (Nos. 1 2, 4 7 9 in group II)
- Non addicts with renal insufficiency not taking phenacetin during survival time determination (11 cases)
- Non-addicts with renal insufficiency taking 1 5 g phenacetin daily during survival time determination (12 cases)

Group III 11 *non*-addicts with renal insufficiency *without* ingestion of phenacetin during the test.

Group IV 12 *non*-addicts *with* ingestion of phenacetin during the test (1 5 g daily)

Table I shows the patients in group I The reason for the phenacetin abuse (generally of many years duration) was headache, migraine, myositis, polyarthritis, osteo-arthrosis and psychopathology All the patients in group II had chronic pyelonephritis. It has not been taken into consideration that this might *primarily* have been due to causes other than the use of phenacetin. One patient had been previously but was no longer

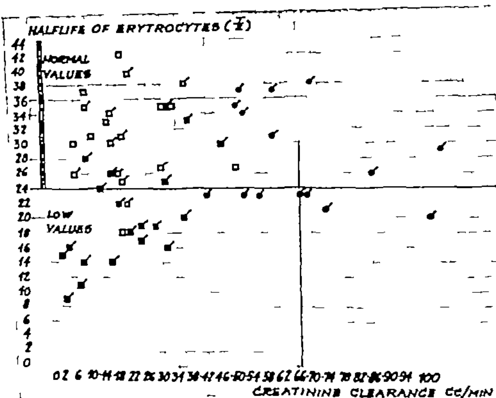


Fig 2 Erythrocyte half-life (T) in relation to creatinine clearance.

Phenacetin addicts without renal insufficiency (15 cases taking 1.5 g phenacetin during survival time determination.

Phenacetin addicts with renal insufficiency (20 cases) taking 1 5 g phenacetin during survival time determination

☐ Non-addicts with renal Insufficiency (9 cases not taking phenacetin during survival time determination.

☐ Non-addicts with renal insufficiency (11 cases) taking 1 5 g phenacetin during survival time determination

Discussion

The -chromium technique is now recognized as a valuable method for determining the erythrocyte survival time in anaemia of varying origin, and particularly also renal insufficiency (Joske et al. (14)).

The results of the present investigations are assembled in fig 1 as regards the relation to serum creatinine, and in fig 2 as regards creatinin clearance. It will be seen clearly that

1 In the present material renal insufficiency alone in itself does not cause reduction in the erythrocyte survival time in cases of very severe uraemia with or without acidosis.

2. The erythrocyte survival time in control patients, i. non-addicts to phenacetin with renal insufficiency is not re

Table IV Erythrocyte survival time and renal function in non-addicts to phenacetin with renal insufficiency with administration of phenacetin

Pt. No.	Sex	Age	Hgb. >80 %	Sulph-hgb. ≦2 %	Reticulocytes <10 ‰	Haptoglobin 35—170 mg %	Renal function			Erythrocyte survival time $\frac{T}{2}$ days 24—36 days
							Sp gr 10³ plus	Creatinine		
								Clearance >67 ml/min.	mg % in serum <1 4 mg %	
1	M	60	71	3.5	10	464	06—11	20.0	3.64	22
2	F	43	80	0 4	10	49	09—14	19.0	3.36	25
3	F	21	55	0 4	27	109	08—12	6.3	6.73	26
4	M	21	73	—	—	15	07—10	18.0	5.65	26
5	M	78	71	2 4	6	124	14—18	30 0	2.70	27
6	F	74	79	0 7	9	221	17—23	15.3	4.52	30
7	F	68	60	0	5	334	09—14	—	3.22	30
8	F	72	87	0.9	7	0	15—16	19 0	1 77	31
9	M	77	75	0 0	4	58	08—13	15.0	4.63	34
10	M	34	48	0.8	2	236	08—11	7.8	10.79	35
11	M	62	112	0 0	8	390	06—11	36.0	2.35	38
12	F	65	70	0 4	8	586	09—15	21.0	1.03	39
		Mean	73 4	0.86	8.7	216.3	10—14	18.9	4.28	30.3

No. 2 had taken 250—300 mg phenacetin daily No. 12 took a sandon tablet occasionally One patient (No. 12) had papillary necrosis.

Of the 16 phenacetin habitués *without* renal insufficiency i. e. with serum creatinine < 1 4 mg % (table I) 8 had creatinine clearance slightly below normal i. e. < 67 ml/min calculated as the average of three days clearance. However all had normal serum creatinine at the time of the survival time determination Nos 2, 3 5 10 13 and 14 had intermittent albuminuria or pyuria Seven had slight reduction of the survival time (see also table I) Grell et al. (9) found normal $\frac{T}{2}$ with a daily phenacetin ingestion of ≦ 1.25 g but a reduction with ingestion of about 2 g daily

In group II the phenacetin habitués with renal insufficiency (table II) the erythrocyte survival time during ingestion of phenacetin was reduced in 15 out of 22 patients. The test was repeated three months later in 5 of these (marked 1 2, 4 7 9 on fig 1) *without* simultaneous ingestion of phenacetin. At that time the erythrocyte survival time was normal or almost normal i. e 23—44—47—40 days as against 9—11—14—16 and 17 days.

The survival time *without* phenacetin in group III patients, the control group of non addicts with renal insufficiency of about the same degree as the phenacetin habitués (table III) was reduced in one instance only (It will be seen that six of this group had taken phenacetin some years previously and therefore the series cannot be said to be completely exact)

All except one in group IV (table IV) also a control group containing 12 non-addicts with renal insufficiency who received phenacetin during the test had normal erythrocyte survival time.

long duration caused by the breakdown products of phenacetin (at any rate alone) should result in nephropathy

Compared to the enormous use (27) and abuse (1 1 23) of phenacetin in this country clinically recognizable "phenacetin nephropathy" is only rarely found. It is also strange that patients with chronic polyarthritis, who for years have taken just as many tablets containing phenacetin as other phenacetin habitués, very seldom — if at all — suffer from kidney disorders as a result (Gsell, (10) Sorensen (30)) There is only one in the present series. Can it be that polyarthritis patients possess a different allergy reserve? Perhaps some persons can develop medicamentous phenacetin allergy more easily than others who eventually develop some kidney disorder as part of their clinical condition.

The deviation in the erythrocyte survival time in relation to the creatinine clearance in fig. 2 might be explained by a varying degree of sensitization such as can be seen, as far as the symptoms are concerned, in other allergic conditions caused by drugs.

This may also be a reason why the phenacetin habitués with pyelonephritis are repeatedly admitted to hospital with symptoms of both acute haemolytic anaemia and acute deterioration of the renal function Apparently where there is greater sensitization as compared with other medicamentous allergic conditions, even quite small daily doses of phenacetin are enough to initiate haemolysis (see Plum's experiments with amidopyrin (28) One or two saridon tablets a day seem to be sufficient In the study of Lorenzen & Schwartz (19) signs of haemolysis could be seen as early as after two to three days ingestion of 2 g phenacetin per day *The use by these patients of phenacetin should be absolutely forbidden* though after so many years of abuse it is doubtless extremely difficult for them to comply with such a ban.

Summary

The study includes 16 phenacetin habitués *without* renal insufficiency (i e. with serum creatinine < 1 4 mg %) 22 phenacetin habitués *with* renal insufficiency and 11 + 12 non-addicts *with* renal insufficiency Determination of erythrocyte survival time was performed in these patients by means of the chromium technique with or without ingestion of 1.5 g phenacetin daily during the test. It was found

1 That in the present material mere renal insufficiency did not itself cause reduction of the erythrocyte survival time, even in those cases with very severe uraemia with or without acidosis.

2. That the erythrocyte survival time in control patients i. e. non-addicts to phenacetin *with* renal insufficiency is not reduced by ingestion of phenacetin during the test.

3 That the erythrocyte survival time in phenacetin habitués with renal insufficiency becomes reduced only if phenacetin is ingested during the test without phenacetin they behave like the control group.

4 That after ingestion of phenacetin by phenacetin addicts, a certain correlation can be found between the degree of renal insufficiency and the reduction in erythrocyte survival time, the greatest reductions being found in the cases with the most severe degree of renal insufficiency

A possible relationship between reduction in erythrocyte survival time and kidney impairment in phenacetin habitués is discussed.

duced by the ingestion of phenacetin during the test.

3 The erythrocyte survival time in phenacetin habitués with renal insufficiency is reduced only if phenacetin is given during the test. Without phenacetin their reaction is the same as that of the patients in the control material

4 When phenacetin is ingested by the phenacetin habitués, there is a certain correlation between the degree of renal insufficiency and the reduction in the erythrocyte survival time, since the greatest reduction is found where there is the most severe degree of renal insufficiency

Comparison of the mean values for the erythrocyte *half life* $\frac{T}{2}$ *in the four groups* of patients shows that the phenacetin addicts (group II) were significantly different from the other groups ($p < 0.001$) while groups I III and IV were not distinguishable from one another

It will be seen from comparison of the *sulphhaemoglobin concentration*, the reticulocytosis and the haptoglobin concentration that the sulphhaemoglobin concentration had a tendency to be greater in the phenacetin addicts and particularly in those with renal insufficiency The reticulocytosis seemed also to be greatest in *that group but here there was a large* scatter The haptoglobin concentration was about the same in all patients, and this value was only determined in a few cases in groups I and III

It cannot be entirely excluded that the reason why the erythrocyte survival time is *generally shorter* in the phenacetin habitués with renal insufficiency (table II) than in the other patients may be that an accumulation of phenacetin may have occurred On the other hand the erythrocyte survival time became normal when no further phenacetin was ingested and several of the patients in group II had only a slight increase in sulphhaemoglobin even though the erythrocyte survival time was considerably reduced.

As mentioned in a previous study it has been demonstrated that there must be a factor in plasma which influences the erythrocyte survival time, and that prednisone reduces the haemolytic effect of that factor thus suggesting a sensitizing *mechanism. The extended studies carried* out show that actually it is a question of phenacetin allergy and that the possible direct damage caused by phenacetin during the determination has little significance for the reduction of the erythrocyte survival time.

Is there then a relationship between this phenacetin allergy and the kidney disorder or is the allergy purely a symptom in phenacetin addicts who for some reason have pyelonephritis? That is to say is there any relationship between phenacetin abuse and nephropathy?

Various hypotheses and surmises have been advanced regarding the origin of the so-called interstitial nephritis

1 *Local* toxic damage to the kidney tissue caused *by the breakdown products* of phenacetin precipitation into the tubuli of methaemoglobin sulphhaemo*globin, etc. (2 9 11 12 23)*

2 Chronic hypoxia in the interstitial kidney tissue *as the result of* methaemoglobinaemia and sulphhaemoglobinaemia *(15)*

3 An allergic condition in the peritubular tissue caused by phenacetin (3 19 21 23)

Generally a relationship has been found between the extent of the damage provoked by phenacetin and the size of dose and duration of ingestion (8 9 15 17 29) However some factor seems to exist which is not consistent with the theory that a simple, toxic damage of

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The Inactivation of Hemolytic Complement by Human γ-Globulin — Sheep Antihuman γ-Globulin Immune Complexes

By

Åke Nordén

with the technical assistance of Anita Mucha

In previous report (Lundqvist and Nordén 1960) the inactivation of hemolytic complement by bovine albumin and rabbit antialbumin serum was described. The fastest inactivation was found when the immune precipitate was formed in slight antibody excess. As the antigen-antibody complexes producing the most extensive tissue-damaging effect, judging from studies of soluble complexes, seem to be formed in a certain range of antigen excess (Ishizaka et al. 1960) tissue destruction potency and the rate of inactivation of hemolytic complement may reach their peaks t different antigen-antibody ratios and thus presumably during different phases of the immunization process. The latter may be assumed to follow a pattern such as antigen dissemination — antigen uptake by cells — antibody formation — formation and elimination of antigen-antibody complexes with antigen excess, at equilibrium and finally with antibody excess. The phase in this sequence of events during which inactivation of hemolytic complement is most extensive may therefore not coincide with the phase of greatest importance as a cause of tissue damage. For the understanding of the role of hemolytic complement in human disease, it seemed necessary t study other antigen-antibody systems.

For the present studies human γ-globulin was selected as the antigen. It has obvious merits because of its clinical association with diseases such as heumatoid arthritis, multiple myeloma, Waldenström macroglobulinemia nd so-called utoimmune disorders.

Submitted for publication August 7 1961

Supported by grant from the Swedish Medical Research Council which is gratefully acknowledged.

Presented in part at the meeting of the Swedish Bacteriology Society in Stockholm Dec. 1960.

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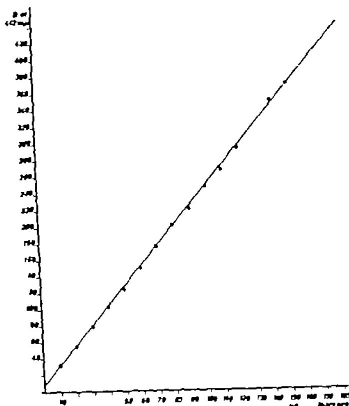


Fig 1 Calibration of O D values obtained with the Nelson (1958) method against μg of protein nitrogen determined by the micro-Kjeldahl method.

Results

The human γ-globulin—anti-γ-globulin precipitin reaction is illustrated by fig 2 The reaction follows in antibody excess and equivalence the formula

Antibody nitrogen precipitated =

$$= 2\,Rx - \frac{R^2}{A}x^2$$

in which the antibody nitrogen antigen nitrogen ratio t equivalence (R) = 3 6 the maximum antibody nitrogen precipitated (A) = 63 μg and the amount of antigen used is = x.

I gel precipitation tests using the Ouchterlony technique (fig 3) precipitation lines were observed between anti-γ-globulin and undiluted human serum but not between anti-γ-globulin and undiluted guinea pig serum. No line was demonstrable between the antiserum and the human γ-globulin solution The concentration employed in the quantitative precipitin reaction was used and this may have been insufficient. It cannot, how ever be excluded that the antibody had been precipitated by the human serum (human complement)

Four lines were observed between the antiserum and undiluted human serum, two f which v ere strong, while the re maining two wer weak. The strong line close to the antiserum showed cleavage at its tails.

Guinea pig serum human serum and human γ-globulin were separated by the

Material and methods

1 *Human γ-globulin*

Purified human γ-globulin was kindly supplied by Kabi, Stockholm. On paper electrophoresis it was found to consist of 96.7 per cent γ-globulin and 3.3 per cent albumin. A solution containing 250 mg per 100 ml of distilled water was prepared. Merthiolate in a final dilution of 1 : 10 000 was added as a preservative.

2 *Antiserum against human γ-globulin*

Human γ-globulin solution was suspended in an equal volume of Freund's complete adjuvant. Ten ml of this mixture was injected subcutaneously into three sheep once a week for ten weeks. Satisfactory antibody formation determined by visible precipitation in a test tube was obtained in the serum from two animals. They were bled to death 8 days after the last injection by canulation of the jugular vein under sedation with chlorpromazine. The antibody content, determined according to Heidelberger and Kendall using a Markham micro-Kjeldahl apparatus (Lundqvist and Nordén 1960), was found to be 500 μg of protein nitrogen per ml in the serum used for the present studies. The serum was clarified by centrifugation at 8—10 000 g for 2 hours in a Pywe refrigerated centrifuge and kept frozen at —50° C. The serum was absorbed twice with human red blood cells of blood group AB Rh-positive.

3 *Serum complement*

Serum was obtained from freshly drawn human and guinea pig blood which had been allowed to clot at room temperature for two hours. It was kept frozen at —50° C until used.

4 *Sensitized sheep cells*

Sensitized sheep cells were obtained and prepared as previously described (Lundqvist and Nordén 1960).

5 *Diluent*

Veronal-buffered saline pH 7.40 was prepared as described by Nordén and Swahn (1961).

6 *Quantitative protein determinations*

The quantitative precipitin reaction was performed as described previously (Lundqvist and Nordén 1960). In the present studies a volume of antiserum of 0.2 ml was used. In addition to the micro-Kjeldahl, the method described by Nielsen (1958) was employed for quantitative determinations of protein in the precipitate.

R e a g e n t s. 5 per cent solution of Na, PO$_4$ · 12 H O in distilled demineralized water. 4 per cent solution of CuSO · 5 H O in distilled demineralized water. 0.5 g of sodium-diethyldithiocarbamate in 5 ml of distilled water.

P r o c e d u r e. The sodium phosphate solution was added to the precipitate to make a final volume of 5 ml. Fifty μl of the copper sulphate was added. The mixture was shaken for 30 minutes and the precipitate formed was removed by centrifugation for 10 minutes. From the supernatant 0.5 ml was pipetted off into a tube with a snugly fitting glass stopper. Five ml of sodium phosphate solution and 0.1 ml of freshly prepared sodium-diethyldithiocarbamate solution was added to the tube. The optical density of the yellow color was measured after 15 minutes in a Beckman DU spectrophotometer at 442 mμ against the phosphate reagent as a blank. A calibration curve is illustrated by fig. 1.

7 *The inactivation of hemolytic complement*

The technical details have been described previously (Lundqvist and Nordén 1960). The volume of complement added to the precipitate was 5 ml. Guinea pig serum was diluted 0.8 → 50 and human serum 1 → 5. In order to exclude any reaction between anti-γ-globulin serum and the γ-globulin present in the serum used as a source of complement activity, the immune precipitate was washed in the cold until free of excess anti-γ-globulin. Before being used the precipitate was broken up mechanically as previously described. Serial determinations of the hemolytic complement activity were performed and the degree of inactivation was expressed as a percentage of the hemolytic activity of the complement at the time when the sample was drawn from the γ-globulin—anti-γ-globulin—complement mixture.

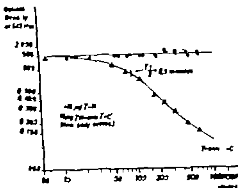


Fig. 5. Inactivation of human hemolytic serum complement by human γ-globulin and antihuman γ-globulin immune complex formed in antibody excess.

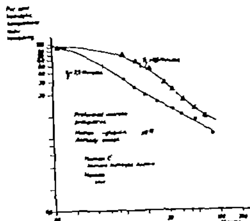


Fig. 6. Inactivation of hemolytic complement.

As seen from fig. 5 no change in the hemolytic activity of complement was observed during the time required for the experiment, in this case 60 to 70 minutes. The presence of γ-globulin corresponding to 15 μg of protein nitrogen did not influence the activity of complement. The hemolytic activity of complement, expressed in terms of the optical density produced by the hemoglobulin released from hemolyzed red cells, was reduced by half within 8.5 minutes.

The rate of the inactivation of complement by the antigen-antibody complex is determined not only by the composition of the immune complex, but may vary with the source of complement as illustrated by fig. 6. In this experiment again 15 μg of γ-globulin was used. Complement was obtained from one case of rheumatoid arthritis complicated by hemolytic anemia and from one case of chronic lymphocytic leukemia also complicated by hemolytic anemia. The complement activities in the two sera were 43.5 and 30.5 fifty per cent hemolytic units per ml. In the first case the half time for inactivation was 8.5 minutes and in the second about 2.5 minutes. The content of γ-globulin in the two sera was 1.32 g per 100 ml and 0.40 g per 100 ml. As seen from the curves the serum contributing the largest amount of additional human γ-globulin showed the slowest inactivation curve. This may represent an effect of antigen excess.

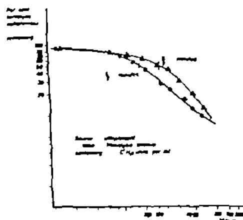


Fig. 7. Inactivation of human complement by preformed and newly formed immune complexes.

These two sera were also found to differ when they were exposed only to anti-γ-globulin. Their complement activity decreased and this was thought to be due

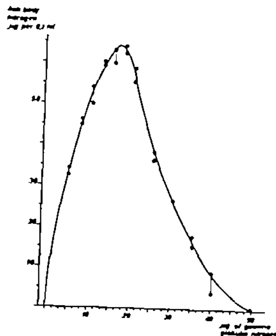


Fig 2 Quantitative precipitin reaction between human γ-globulin and sheep antihuman γ-globulin antibody Range of three determinations indicated.

Sheep anti-human gamma globulin and

1 Guinea pig serum



2 Human serum

3 Guinea pig serum



4 Human gamma globulin

Fig 4 Immune electrophoresis.

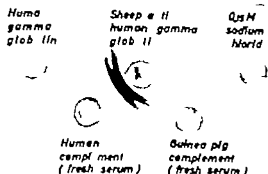


Fig 3 Gel precipitation according t Ouchterlony

micro-immune electrophoresis technique and exposed to the antihuman γ-globulin serum as illustrated by fig 4 No precipitation was observed with guinea pig serum. Multiple lines were obtained with whole human serum and a single line with human γ-globulin.

In the reactions to be described the preformed precipitate represented according to these tests a simple immune system while in the reactions between anti γ-globulin and the human serum used as a source of complement multiple systems were involved in the inactivation of complement

1 *The inactivation of human complement*

The inactivation of human complement by preformed γ-globulin — anti γ-globulin precipitate formed in slight antibody excess is illustrated by fig 5 The actual optical density readings for the degree of hemolysis have been plotted for the immune precipitate with added complement, and for controls containing complement only and complement plus the amount of human γ-globulin used for the formation of precipitate In addition the reaction between anti γ-globulin and complement was also followed The results are described later when preformed and newly formed antigen antibody complexes are compared.

Table I Distribution of serum protein fractions

	Albumin	α 1 globulin	α 2	β	γ
	In g per 100 ml				
Guinea pig serum	2.41	0.37	0.55	0.58	0.39
Sheep anti-γ globulin	1.21	0.51	0.33	0.56	3.69
	in per cent of the protein content				
Human γ-globulin	3.3	—	—	—	96.7

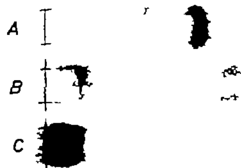


Fig 9. Paper electrophoresis. A. Guinea pig serum. B. Sheep antihuman γ-globulin serum. C. Human γ-globulin.

cess. The results were taken to suggest that small antigen-antibody complexes react more rapidly with complement than do larger aggregates but that after some delay the final inactivation capacity in the latter case may be just as large. The assumption is being made that during the delay the larger complexes are being dissociated into smaller units.

2. *The inactivation of guinea pig complement*

The paper electrophoretic patterns of the guinea pig serum, the sheep antihuman-γ-globulin serum and the human γ-globulin are illustrated by fig 9 and table I. We were surprised to find a very low content of γ-globulin in the guinea pig serum. R tquist et. al (1956) found however in 1 normal guinea pigs γ-globulin values of 0.51 ± 0.031 g per 100 ml. Apparently the γ-globuli content in guinea pig serum is normally low. This should be taken into consideration when the low resistance t nfections in this species is being discussed. The sheep serum had n increased content of γ-globulin nd very low albumin level. No edema w seen in these animals. The lymph nodes were generally enlarged. Microscopically they showed an increased number of plasma cells. In a few nodes giant cells and focal necrosis were observ ed. The njections with Freund s adjuvant had produced a series of sterile abscesses under the skin of the back. In addition the animals had been lactating The human γ-globul n sample was found by paper electrophoresis to contain 3.3 per cent moving as albumin.

As already mentioned no precipitin lines were discovered in the Ouchterlony test or by immune electrophoresis between guinea pig serum and sheep antihuman-γ-globulin. This could be due to the absence of a cross reaction between the antiserum against human γ-globulin and guinea pig γ-globulin, but it might also be caused by the lack of sensitivity inherent n the gel precipitation technique. The experiment illustrated by fig 10 made use of a preformed human γ-globulin—anti-γ-globulin precipitate and the reaction between antihuman-γ-globulin and guinea pig serum. The results were similar to those previously described, the nactivation taking place in the test using the antiserum with guinea pig com plement had more rapid initial phase than the inactivation produced by the preformed precipitate. The results were taken to uggest a cross reaction between

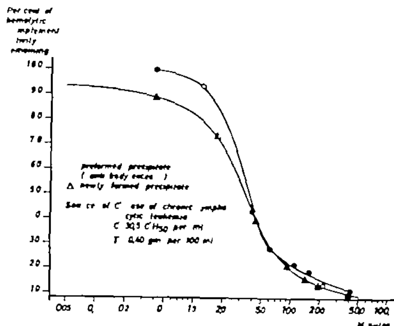


Fig 8. Inactivation of human complement by preformed and newly formed immune complexes.

to the reaction between anti γ-globulin and the γ-globulin present in the serum containing complement. Thus in these experiments complement was present when the antigen-antibody reaction took place that is, in statu nascendi of the immune complex. It has previously been assumed in quantitative studies of the complement protein uptake on immune precipitates that complement might interact between antigen-antibody complexes (Lundqvist and Nordén 1958) If so one would expect a more rapid inactivation of complement in a reaction system in which antigen and antibody react in the presence of complement In fig 7 such a reaction with complement from the patient with rheumatoid arthritis and hemolytic anemia is illustrated In the system in which anti γ-globulin reacts with the serum containing complement, there is a suggestion of a more rapid initial phase than in the reaction with preformed precipitate Fig 8 illustrates the same reaction using complement obtained from the case of chronic lymphocytic leukemia and hemolytic anemia. Here the reaction between complement and the preformed γ-globulin—anti γ-globulin complex proceeds at a clearly slower initial rate. This experiment also shows that the two curves reach a plateau suggesting that the final inactivation of complement is comparable. The difference in the two reaction systems is thus hardly due to a reaction between anti γ-globulin and parts of the hemolytic complement or to any appreciable difference in the amount of available antigen-antibody complex. In the latter experiment the source of complement contained less γ-globulin than in the experiment illustrated by fig 7 This may explain the more rapid initial phase, there being presumably less inhibition by excess γ-globulin

In the two series of experiments just mentioned the antigen-antibody complex used for the inactivation of complement had been formed in slight antibody excess. The difference in initial reactivity between preformed precipitate and the reaction assumed to represent an in statu nascendi situation was also found with immune complexes formed in antigen ex

Table II. Times required for the inactivation of hemolytic guinea pig complement to fifty per cent of its original activity by human γ-globulin — anti-γ-globulin complexes formed at different levels of antigen with constant amount of antibody

Antigen Human gamma globulin nitrogen µg	Time min.	Excess Antibody Ab Antigen Ag
1	39	Ab
5	30	Ab
10	27.5	Ab
20	43	—
30	46	Ag
40	46	Ag
50	51	Ag

inactivation. In table II the half time values for the inactivation produced with varying amounts of antigen have been recorded. On the antigen excess side the differences in the amount of antigen used were presumably not large enough to influence the rate in the same way as on the antibody excess side. This agrees with the findings in the bovine albumin—anti-albumin system in which, however the reactions proceeded t much higher rates (Lundqvist and Nordén 1960). The reason may n part be that in these studies the antigen-antibody interaction was given only one minute before the complement was added The similarity between the two systems may also be illustrated by the elation between the shortest inactivation tim nd th inactivation time for the precipitat formed with the amount of ntigen corresponding to the ordinat = 0 on the precipitin curve at the antigen excess side. This figure was found to be 27.5 31 in the present series nd 4 8 in the lbumin sy tem, thus pproximately 1 in both.

Discussion

The present studies were intended to explore the background for in vivo changes in the complement activity of serum. It was originally assumed that a decrease in the hemolytic activity might suggest that complement had been used up in excess of the production. As means of determining the total amount of complement being produced or destroyed in vivo by turn-over studies are not yet technically available, a kinetic in vitro method was used to investigate the parameters for the most rapid inactivation of hemolytic complement by antigen — antibody complexes.

The human γ-globulin—antihuman-γ-globulin system was found to inactivate hemolytic complement most rapidly when the antigen—antibody complexes were formed in antibody excess. In the series of experiments performed, the most rapid inactivation of guinea pig complement took place with a quantity of antigen amounting to half of that required for optimal precipitation of antibody This accords with our previous findings with bovine albumin — antialbumin complexes. The inactivation times with the γ-globulin system were longer than w th th albumin system, but the proportions between inactivation times found with corresponding types of immun complexes in th two systems were of the same order Th difference may be related to the physical —chemical characteristics of the antigens or the antigen-antibody complexes formed. It may however be related t the difference in the technique employed. In the albumin system w th a rapid inactivation the complement was, except for the first minute, present during the antigen-antibody nteraction, while in the present studies a preformed precipitat was used to which the complement was later added.

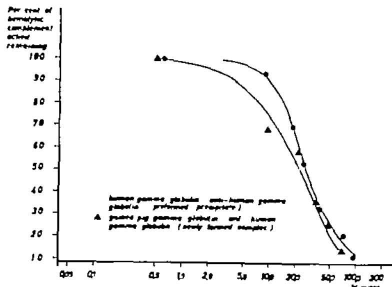


Fig 10. Inactivation of guinea pig complement by human γ-globulin—anti-γ-globulin complex and by the reaction between guinea pig γ-globulin and antihuman γ-globulin.

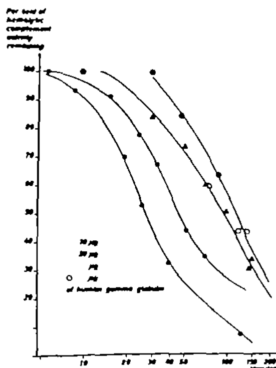


Fig. 11 Inactivation of guinea pig complement by preformed immune complexes formed in varying degrees of antibody excess.

the antiserum and guinea pig γ-globulin. A non immune aggregation of proteins in the system leading to a loss of hemolytic effect could not be excluded but seemed a less likely explanation. If the conclusion is correct then the present system is a more sensitive means of detecting antigen—antibody reactions reacting with complement than the gel precipitation technique — for practical purposes it is obviously much more laborious and therefore less useful.

The effect of changing the antigen—antibody ratios on the inactivation of guinea pig complement by preformed human γ-globulin—antihuman γ-globulin precipitates was then studied. Fig 11 illustrates the inactivation curves obtained with amounts of antigen representing equivalent proportions and increasing amounts of antibody excess The fastest reaction was found when 10 μg of γ-globulin protein nitrogen was used representing half the amount required for optimal proportions for precipitation Increasing or decreasing the amount of antigen produced slower reactions When 5 μg and 1 μg of γ-globulin were used a considerable lag phase was noted amounting to about 30 minutes with 1 μg Nevertheless as far as it was possible to follow the inactivation, the final effect was of a comparable magnitude — approaching complete

Table II Times required for the inactivation of hemolytic guinea pig complement to fifty per cent of its original activity by human γ-globulin — anti-γ-globulin complexes formed at different levels of antigen with constant amount of antibody

Antigen Human gamma globulin nitrogen μg	Time min.	Excess Antibody Ab Antigen Ag
1	80	Ab
5	90	Ab
10	27.5	Ab
20	43	—
30	46	Ag
40	46	Ag
50	51	Ag

inactivation. In table II the half time values for the inactivation produced with varying amounts of antigen have been recorded. On the antigen excess side the differences in the amou t of antigen used were presumably not large enough to in fluence the rate in the same way as on the antibody excess side. This agrees with the findings in the bovine albumin—antialbumin system in which, however the reactions proceeded t much higher rates (Lundqvist and Nordén 1960) The eason may in part be that in these studies the antigen-antibody interaction was given only one minute before th com plement was added. The similarity be tween the two systems may also be illustrated by the elation between the shortest inactivation time nd the inactivation time for the precipitate formed with the amount of antigen corresponding to the ordinat = 0 on the precipitin curve at the antigen excess side This figure was found to be 27.5 51 in the present series and 4 8 in the albumin system thus p- proximately 1 2 in both.

Discussion

The present studies were intended to explore the background for in vivo changes in the complement activity of serum. It was originally assumed that a decrease in the hemolytic activity might suggest that complement had been used up in excess of the production. As means of determining the total amount of complement being produced or destroyed in vivo by turn-over studies are not yet technically available, a kinetic in vitro method was used to investigate the parameters for the most rapid inactivation of hemolytic complement by antigen—antibody complexes.

The human γ-globulin—antihuman-γ-globulin system was found to inactivate hemolytic complement most rapidly when the antigen—antibody complexes were formed in antibody excess. In the series of experiments performed, the most rapid inactivation of guinea pig complement took place with a quantity of antigen amounting to half of that required for optimal precipitation of antibody This accords with our previous findings with bovine albumin — antialbumin complexes. The inactivation times w th the γ-globulin system were longer than with the albumin system, but the proportions between inactivation times found with correspond ing types of immune complexes in the two systems were of the same order Th d fference may be related to the physical —chemical characteristics of the antigens or the antigen-antibody complexes formed. It may however be related to the differ ence in the technique employed. In the lbumin system with a rapid nactivation the complement was, except for the first minute present during the antigen-antibody interaction while in the present studies a preformed precipitate was used t which the complement was later added.

Using the same type of antigen anti body complexes it was found that different human sera were inactivated at different rates. A possible explanation seemed to be the effect of additional γ-globulin contained in the human serum used as complement. This might produce an antigen excess effect, thus slowing down the rate of inactivation.

The type of immune aggregation is known from the studies of Osler et al. (1955) to be of importance for the inactivation of complement. In the present studies the rate of inactivation produced by preformed, washed and then mechanically broken up complexes was compared with the rate of inactivation produced by complexes assumed to be formed between the anti γ-globulin serum and the γ-globulin of the complement in the presence of active complement i e in statu nascendi It was found that in the latter case a more rapid initial inactivation took place in systems giving comparable final hemolytic inactivation In fact with some preformed complexes there was such a latency period that it may be asked whether the precipitable complexes have to dissociate into soluble complexes before the reaction with complement takes place

Attempts to relate the present findings to in vivo pathogenetic mechanisms are probably hazardous. During the immunization process against any exogenous or endogenous antigen, an initial phase characterized by antigen excess exists Ishizaka et al. (1960) demonstrated that immune complexes of different composition had different degrees of skin irritating effect in the guinea pig Complexes containing two antigen molecules and one antibody (Ag.Ab.Ag) had no skin-irritating effect, while complexes containing two or more antibody molecules were skin irritating (Ag.Ab.Ag.Ab.Ag) Ishizaka et al. (1960) assumed the antibody — i e. the γ-globulin — to be changed during the reaction with the antigen to form a toxic configuration The role of complement in the formation of skin-damaging anaphylatoxin" was studied by Osler et al (1959) who found the reaction to be dependent on the availability of the C'3 component of complement.

From these studies one might expect some correlation between the complement inactivating capacity and the tissue damaging effect of antigen antibody complexes. More information about the biological effect of antigen-antibody complexes formed in antibody excess seems to be needed

Summary

The inactivation of human and guinea pig complement by human γ-globulin and immune antiserum produced in sheep, was studied by kinetic techniques. Immune precipitates formed in antibody excess inactivated complement most rapidly The reaction between anti γ-globulin and the γ-globulin of the serum used as a source of complement showed a more rapid initial inactivation of complement than did preformed immune precipitates.

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From the Department of Medicine (Head H. Malmros, M. D.)
University Hospital, Lund, Sweden

Studies on Inactivation of Complement by Antigen-Antibody Complex (III)

The Inactivation of Hemolytic Complement by Heated Human γ-Globulin—Anti-γ-Globulin Complexes

By

Åke Nordén

with the technical assistance of Anita Mucchi

It has frequently been observed that sera with a high content of γ-globulin will inactivate hemolytic complement. It has been suggested that sera with high anti-complementary activity might contain soluble antigen-antibody complexes, i.e. γ-globulin — anti-γ-globulin complexes. The possibility was discussed by Marcus (1960) who thought it, however rather unlikely.

A characteristic feature of most anti-complementary sera seems to be that the anticomplementary activity increases following heating at 56 C — that is, following the usual procedure for the inactivation of complement in complement fixation tests.

In the present studies the inactivation of hemolytic guinea pig serum complement by immune γ-globulin — anti-γ-globulin precipitates heated at 56 C for 60 minutes has been investigated. Our results indicate that γ-globulin — anti-γ-globulin complexes have a lower capacity to inactivate hemolytic complement after they have been heated.

Material and methods

The technique used was the same as that described in paper II (Nordén, p. 135). The preformed immune precipitates were incubated for 60 minutes at 37 C and then for 7 days at 2—3° C under strictly sterile conditions. They were washed by centrifugation in a refrigerated centrifuge (Pyw) and resuspended in sterile saline. Following the last centrifugation the sediment was thoroughly broken up by the vibrations of a rotating metal rod. The precipitate was left at 2—3° C overnight. Heating of the precipitate at 56 C for 60 minutes was performed the following morning, after which the inactivation experiments with complement were started.

Submitted for publication August 7, 1961

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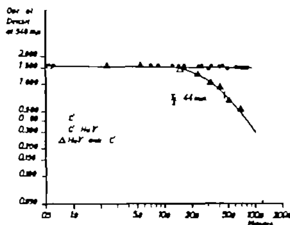


Fig 1 Inactivation of guinea pig hemolytic complement by human γ-globulin — anti-γ-globulin complex.

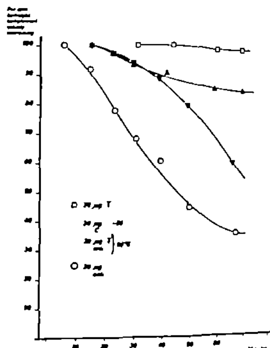


Fig 2 Inactivation of guinea pig complement by heated and unheated immune precipitate (equivalent proportions)

Results

A typical experiment is illustrated by fig 1 In the controls containing guinea pig complement and human γ-globulin the γ-globulin did not affect the complement activity Inactivation produced by the precipitate became measureable in this experiment after 15 to 20 minutes, and a fifty per cent reduction of the optical density was recorded after 44 minutes. The immune precipitate was in this case produced with 20 µg of human γ-globulin protein nitrogen as the antigen representing equivalent proportions to the antibody content or a minute excess of antigen.

1 *The inactivation of guinea pig complement by heated and unheated immune complex formed in the equivalence zone*

Fig 2 illustrates the results obtained with precipitates formed by the use of 20 µg of γ-globulin protein nitrogen. The hemolytic activity of the complement control in this and the following experiments at any given time during the experiment is given the value 100 During the 60 to 70 minutes of this experiment the immune complex heated to 56 C produced less inactivation of the hemolytic complement than did the unheated precipitate. The reaction had a slower start and the time required for a reduction of the hemolytic complement level by 50 per cent was 73 minutes as compared to 45 minutes when unheated precipitate was used The effect of heated and unheated human γ-globulin on guinea pig complement is also included. Heated γ-globulin inactivated guinea pig complement to some extent but unheated it had only a very slight effect.

2. *The inactivation of guinea pig complement by heated and unheated immune complex formed in antibody excess*

Fig 3 illustrates the results when 5 µg of γ-globulin protein nitrogen was used as the antigen representing antibody excess. The findings were closely similar to those observed with immune precipitate

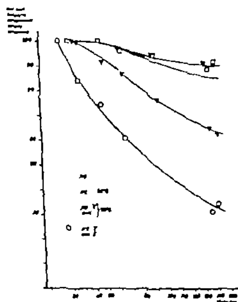


Fig. 3. Inactivation of guinea pig complement by heated and unheated immune precipitate (antibody excess).

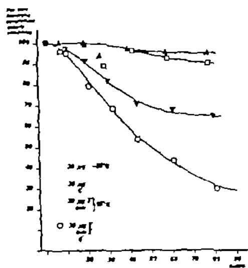


Fig. 4. Inactivation of guinea pig complement by heated and unheated immune precipitate (antigen excess)

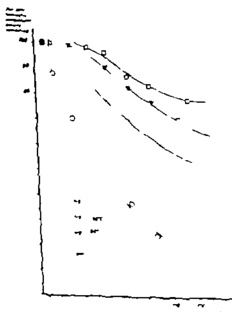


Fig. 5. Inactivation of guinea pig complement by heated and unheated immune precipitate (antigen excess

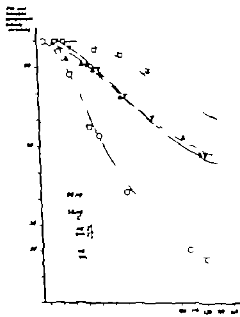


Fig. 6. Inactivation of guinea pig complement by heated and unheated immune precipitate (antigen excess

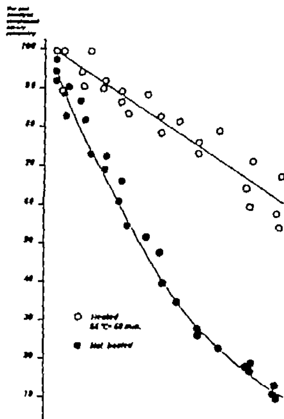


Fig 7 Inactivation of guinea pig complement by heated and unheated antihuman γ-globulin.

formed in the equivalence zone. Unheated immune complex inactivated complement throughout to a greater extent — the time for 50 per cent hemolytic reduction was 86 minutes, while with heated complex this point was never reached within the 140 minutes of the experiment.

3 The inactivation of guinea pig complement by heated and unheated immune complex formed in antigen excess

Figs. 4 5 and 6 illustrate the results obtained when immune precipitates were produced with increasing amounts of antigen excess. The same results were consistently observed — unheated precipitate inactivated complement faster and to a greater extent than the heated precipitate.

4 The effect of heating anti-γ-globulin on the inactivation of complement

Sheep antihuman γ-globulin was heated for 60 minutes at 56 C and mixed with guinea pig complement, and the hemolytic effect was followed as in the previous experiments. As seen from fig 7, heated anti γ-globulin inactivated complement when reacting with the γ-globulin of complement to a lesser degree and at a slower rate than did unheated anti γ-globulin The results were thus in agreement with those obtained when preformed immune precipitates were studied after heating and unheated

5 The effect of heating human γ-globulin on the inactivation of complement

Human γ-globulin used as the antigen in the present studies was heated for 60 minutes at 56 C and mixed with guinea pig complement. The hemolytic activity was followed in serial samples. From figs. 2—6 it is apparent that heating samples containing 5 to 50 μg of γ-globulin protein nitrogen regularly increased the complement inactivating effect. The results were thus suggestive of an opposite effect of heating on non-antibody γ-globulin as compared to the effect on antibody γ-globulin when tested with the complement inactivation technique

Discussion

The present studies indicate that heated γ-globulin — anti-γ-globulin precipitate formed in antibody excess, at equivalent proportions or in antigen excess has a decreased capacity to inactivate guinea pig hemolytic complement Assuming the same to be true when soluble complexes are present the experimental results may argue against the theory that human sera showing an increased anticomplementary activity after heat in-

activation are anti-complementary because the γ-globulin consists of a mixture of anti-γ-globulin—γ-globulin immune complexes. With the present technique, heating of γ-globulin was found to produce an increased anti-complementary effect.

Heat is known to damage the antibody in weak antisera (Kabat and Mayer 1948). The present studies of γ-globulin as the antigen and also as the antibody indicate the difference in behaviour on heating to 56° C between non-antibody γ-globulin and antibody γ-globulin. Ishizaka and Campbell (1959) found that the formation of soluble antigen-antibody complexes was accompanied by an increase in levorotation. The change was only observed when skin-reactive immune complexes were formed. It was assumed that the toxicity of soluble immune complexes was d e to molecular changes brought about in the *antibody* molecules. Ishizaka and Ishizaka (1960) found that aggregates of γ-globulin produced by treatment with urea and mercaptoethanol or coupling with bis-diazotized benzidine inactivated complement irrespective of the method used for aggregate formation. The findings were interpreted t suggest that the ability to fix complement was due not to a molecular change in the γ-globulin structure prior to the aggregate formation but to the interaction between γ-globulin molecules. Within the scope of the present studies, our results suggest that heating at 56° C for 60 minutes inactivates the antibody γ-globulin as regards its capacity to form precipitable complexes and thereby reduces the inactivation effect on complement, while heating of non-antibody γ-globulin produces aggregates which apparently acquire a capacity to inactivate complement.

Heating at different levels was not attempted in the present studies. Muschel et al. (1961) found that the complement fixing capacity of sera from healthy individuals and from patients with systemic lupus erythematosus containing antibodies against rabbit liver was less after heating to 65 C than after heating to 56° C. Maurer and Thorpe (1960) found that heating of sera to 7 C prevented the formation of precipitate with specific antigen but did not destroy the complement fixing ability. This was, however lost following heating to 85 C. Sera heated to 75 C could still react in passive cutaneous anaphylaxis reactions.

Increased amounts of γ-globulin in various disease states have in many instances been held to suggest immunization. The studies by Campbell and co-workers bring up the possibilities of tissue damage sometimes induced by antibody-aggregation of γ-globulin and sometimes by aggregations induced by other mechanisms as may be the case in the presence of macro-globulins. In both instances the experimental evidence points to the fixation of complement being a characteristic of such biologically active aggregates. In lupus erythematosus a characteristic feature is the low level of serum complement, while in rheumatoid arthritis serum complement is frequently found to be increased or normal but seldom decreased (Nordén unpublished observations). Serum from patients with rheumatoid arthritis contains a γ-globulin or perhaps several γ-globulin components capable of reacting by precipitation with human γ-globulin. The precipitation reaction produced with the so-called rheumatoid arthritis factor is increased following heating at 63 C (Epstein et al. 1957). Winblad (1960) has questioned whether the reaction between rheumatoid arthritis

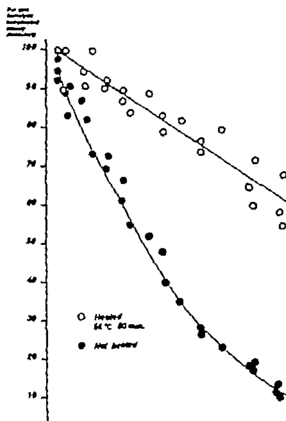


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factor and the patient's own γ-globulin might occur. If the findings of no decrease of serum complement may be interpreted to suggest that the γ-globulin — rheumatoid arthritis factor system does not inactivate complement, one would not expect the presence of *increased* levels of γ-globulin in the serum of patients with rheumatoid arthritis to be of the same pathogenetic importance as for instance in cases of disseminated lupus erythematosus. *Waldenström (1961)* reported that in a series of 34 cases with lupus erythematosus, anti-complementary activity was frequently observed in the patients' serum. The same was true in sera obtained from cases of *hyperglobulin*emia, chronic sialoadenitis, multiple myeloma and some cases of cirrhosis of the liver. In this series of patients one would be tempted to assume as a possible pathogenetic mechanism aggregate formation of γ-globulins to "toxic configurations" with a tissue damaging capacity. In some cases immune mechanisms may be responsible, in others the aggregates may be a consequence of the chemical — physical characteristics of the protein.

Summary

The anti-complementary effect of some human sera is known to increase following heating to 56° C. The present studies attempted to explore the effect of heating immune *γ-globulin — anti γ-globulin* complexes on the velocity of the complement inactivation reaction. Heating of complexes formed in antibody excess, at equivalent proportions and in antigen excess regularly reduced the rate of complement inactivation. Heating of anti γ-globulin alone had the same effect, while heating γ-globulin, i. e. the *antigen*, had the opposite effect and increased the *anticomplementary* effect. The findings may perhaps be explained by the assumption *that antibody is damaged by heating* while γ-globulin itself is aggregated by heating, leading in the first instance to a decrease and in the latter to an increase in complement fixation.

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Identical Changes in Renal Excretion Pattern Induced by Angiotensin and Orthostasis in Normal and Adrenalectomized Subjects

By

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Following massive hemorrhage (1, 2) and in severe heart failure (3) a high concentration of renin has been demonstrated in systemic blood or in renal venous blood. The renal humoral pressure mechanism was considered to represent a compensatory measure.

On the other hand Starling (4) observed a renal retention of "fluid" in both conditions. He regarded the renal response as part of circulatory homeostasis. A retention of water, sodium and chloride in the presence of an almost normal excretion of other urine constituents is observed in all conditions leading to an insufficient filling of the arterial system (5, 6, 7). The excretion pattern is characteristic. The sodium and water retention is often accompanied by a less significant retention of potassium; the output of creatinine is as a rule not, or only slightly depressed. The pattern is identical in subjects with and without adreno-cortical function (8, 9).

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Since in normal man angiotensin causes a significant water and sodium retention (10, 11, 12, 13) the possibility that the circulatory anti-diuresis and anti-natriuresis are mediated by the renin-angiotensin system has to be considered. However, the observation of Genest et al. (14) and Laragh et al. (16) that exogenous angiotensin is a powerful stimulus for aldosterone secretion, suggested that it is the increased secretion of this hormone which is responsible for the sodium retention observed during angiotensin infusions.

Material and methods

The effect of intravenous angiotensin on the urinary excretion of water, sodium, potassium, creatinine and para-amino hippurate was recorded in two patients who underwent total bilateral adrenalectomy for Cushing's syndrome, in one patient who had been subjected to the same operation for severe essential hypertension and in one patient with

Addison's disease. Cortisone therapy was continued on the day of the experiment. Glomerular filtration rate was estimated by the endogenous creatinine clearance, using De Vries and Van Daatselaar's method (17) for the estimation of true creatinine; para amino hippurate clearance was determined by standard methods. Previous investigations in the patient with Addison's disease had shown that ACTH did not affect water, sodium or potassium excretion. With Neher and Wettstein's method (18) no aldosterone could be demonstrated in 24 hours urine of any of the four patients.

Furthermore, in normal subjects and in two patients with essential hypertension the effect of angiotensin on the renal excretion pattern was compared with that of aldosterone and noradrenaline and with the effect of orthostasis. The dosages employed were small in order better to reproduce the possible physiological action of these agents. The dose of noradrenaline was comparable with that of angiotensin in its effect on the bloodpressure in recumbent subjects. The anti-diuresis and the anti-natriuresis associated with orthostasis was chosen for comparison since it represents the renal excretion pattern characteristic of mild, acute circulatory insufficiency (8).

Two methods of study were used.

A. According to Lewis et al. (19) the patient drank every half hour a solution containing 200 ml water and 130 mg sodium chloride and the urine was collected every half hour. After three control periods angiotensin was given intravenously (0.5 γ/min) during two or three half hour periods. These were followed by three other control periods.

B. The two patients with essential hypertension were studied during a four-week period on a strictly standardized diet, during which every three hours identical amounts of food, water and sodium chloride (5 g per 24 hours) were supplied. The patients stayed in bed. The urine was collected every three hours. On the experimental days, separated from each other by one or more control days, the patient received angiotensin, noradrenaline or aldosterone intravenously from 9 a. m. to 3 p. m. On another experimental day the patient was ambulant during the same six hour period. The intravenous infusions were given with a continuous infusion set, the fluid load was kept low (12 ml per hour) and dietary fluid intake adjusted accordingly.

The effect of these drugs and of orthostasis was evaluated against the background of the normal diurnal rhythm by comparing the three hourly excretion of water, sodium and potassium during an experimental day with the average excretion during identical periods on two control days, these being the day preceding and the day following the experimental day. This is graphically represented in figures 3 and 4 in the following manner: Retention on the day of the experiment in comparison with the excretion on the control days is represented by white areas, excess excretion on the day of the experiment is represented by black areas.

The synthetic angiotensin, Val₅ Angiotensin II – Asp – β-Amide, and d,l-aldosterone used in this investigation were kindly supplied by Ciba, Arnhem Basel.

Results

I. *Effect of angiotensin in patients without endogenous aldosterone production*

In three of the four patients angiotensin in a relatively low dosage provoked a significant reduction in the output of water and sodium and an almost comparable reduction in potassium output. In patient I there was only a slight reduction in sodium excretion but after the end of the angiotensin infusion there was a significant rise in sodium and water output (fig. 1).

The sharp reduction in diuresis during angiotension infusion and the steep rise in water output after ending the infusion produce significant "dead-space" effects (9). Both the creatinine and the para-amino hippurate clearance must have been influenced by this factor, the influence on the filtration fraction however being negligible.

A "dead-space" effect is at least partly responsible for the reduction in creatinine output during angiotensin infusion and for the excessive rise in the rebound period. This phenomenon, leading to un-

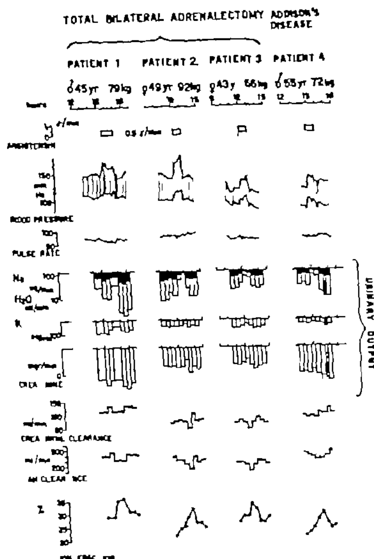


Fig. 1. The effect of intravenous angiotensin on bloodpressure, renal excretion of water (white columns), sodium (black columns), potassium and creatinine and on renal haemodynamics (creatinine and para-amino-hippuric acid clearance, filtration fraction) in patients without functioning adrenocortical tissue.

real variations in the creatinine clearance could be demonstrated in all four patients, but was most pronounced in patients 2 and 3. It should be noted, however that water and sodium output during these periods were reduced much more than was creatinine output.

Owing to these factors a slight reduction in the absolute value of the creatinine clearance cannot be ruled out. The concomitant constant rise of the filtration fraction, however indicates a real and pronounced reduction in the para-amino hippurate clearance, observed in all four patients.

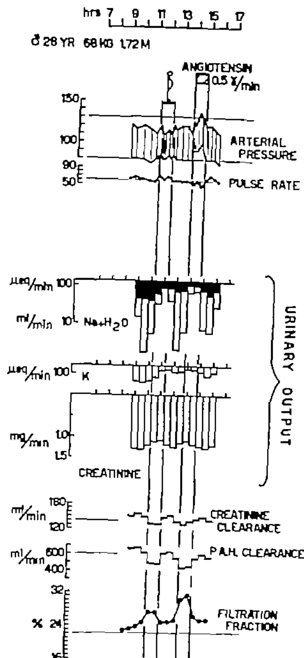


Fig 2 Comparison of renal excretion patterns and renal haemodynamics during orthostatic oliguria (quiet standing) and during intravenous infusion of angiotensin in a normal man (sodium-excretion = black columns water diuresis = whit col-umns)

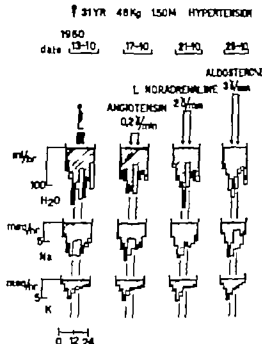


Fig 3. Effect of orthostasis and of various hormones on 24 hour excretion pattern during a regimen of 8 equal three-hourly feedings. The pattern of an experimental day is projected on the a eraged pattern of the preceding and the following control day White columns represent retention, black columns excess excretion on the experimental day Almost constant excretion pattern of sodium and potassium on four a eraged control days. Retention of sodium and potassium of similar type induced by orthostasis and angiotensin, which can be distinguished from the esponse t aldosterone.

II *Effect of angiotensin in comparison with that of quiet standing during short term experiments*

The results of quiet standing and of angiotensin infusion in a normal male student, shown in fig 2 are representative of this group the results in the other subjects being comparable.

Both orthostasis and angiotensin lead to almost identical changes in water sodium and potassium output, in creatinine and para amino hippurate clearance and in the filtration fraction

III *Effect of angiotensin in comparison with that of noradrenaline aldosterone and orthostasis during long term experiments*

The results of the investigations during the standardized diet are plotted in fig 3 and 4

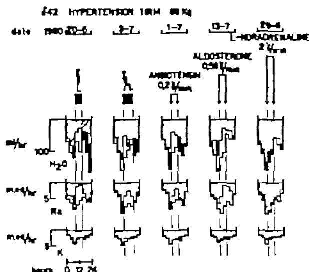


Fig. 4. See legend fig. 3.

During infusion of angiotensin, given in a very low dosage (0.2 γ/min) water and sodium were retained. Moreover a slight potassium retention was noticed in the first patient (fig. 3). The excretion pattern is strikingly similar to the orthostatic anti-diuresis and anti-natriuresis, which is also accompanied by a slight potassium retention. There is an immediate rebound both after stopping angiotensin infusion and after resuming recumbency.

On the other hand the infusion of aldosterone produces an excretion pattern characterized by a sodium retention, still present six hours after its administration. Moreover aldosterone has a tendency to enhance potassium excretion.

Noradrenaline gives variable results: no characteristic pattern can be discerned.

Discussion

The water and sodium-retaining effect of angiotensin in patients without functioning adrenals shows that the renal response is not or not only mediated by aldosterone. The concomitant rise in aldosterone secretion in the presence of functioning adrenals, as shown by Genest et al. (14) and Laragh et al. (16) might be regarded as a parallel effect of angiotensin. It may be produced either by direct stimulation of adreno-cortical tissue, or as a response to the reduction of the adrenal circulation. This reduction results from the vasoconstrictive action of angiotensin which appears especially to affect the splanchnic area (12, 20).

The different effect of angiotensin and aldosterone on the urinary excretion patterns of water, sodium and potassium supports the view that the sodium-retaining action of angiotensin works independently of and differently from that of aldosterone.

The similarity between the effect of angiotensin and the pattern of orthostatic water, sodium and potassium retention suggests a possible role of the renin-angiotensin system in circulatory water and salt retention. This is in accordance with the observations that in conditions associated with an insufficient filling of the arterial system, there exists a hyperactivity of the renin-angiotensin system.

The effect of noradrenaline on the excretion pattern of water sodium and potassium suggests that this agent does not play an intermediary role in orthostatic anti-diuresis and anti natriuresis.

Summary

1 Angiotensin induces a similar renal water and sodium retention in normal subjects and in patients without adrenocortical tissue. Hence the sodium retention is not or not mainly mediated by an increased aldosterone secretion.

2 The renal excretion pattern concerning water sodium potassium creatinine and para amino hippurate caused by a low infusion dosage of angiotensin is similar to the pattern seen in orthostatic antidiuresis. The pattern is different from that produced by the infusion of aldosterone or noradrenaline.

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Amyloidosis of the Heart

By

F. S. P. van Buchem, E. Mandema and A. Arends

In the years before 1940 secondary amyloid was the form of amyloidosis most frequently described. This affection is due to chronic infectious diseases, especially if these are complicated by tissular degeneration (tuberculosis, bronchiectasis, osteomyelitis, ulcerative colitis etc.) In these cases the heart is usually not involved or only to a very slight degree (Dahlin 1949). In contrast to paramyloidosis, to which increasing attention has been given in recent years and in which, as a rule, the heart is one of the most important localizations. According to Higgins (1950) about 56 % of patients with paramyloidosis die of cardiac involvement. Paramyloidosis may be present without indications for another disease and it may also occur in multiple myeloma or macroglobulinaemia. It is often difficult to make the differential diagnosis between primary amyloidosis and amyloid deposition in multiple myeloma (Mandema 1956). one is even inclined to regard most if not all cases of "primary amyloid" either as myelomatosis or as a variant of the clinical syndromes that are based on primary plasma cell proliferation (Propp et al. 1954, Godal 1958, Osserman 1959). At present we will leave it an open question which of our patients suffered from primary amyloidosis, and which from multiple myeloma complicated by amyloidosis. We shall denote the amyloid deposition in our patients by the more or less neutral term paramyloidosis. As we are still almost powerless with regard to the treatment of the two conditions, the distinction between the two is not yet of great significance for the patient. This by no means relieves us of the duty to try to differentiate between these two clinical pictures as adequately as possible, since in future, we may have a better therapy at our disposal.

The following report on our observations in six patients, who all had, besides other localizations, amyloid deposition in the heart.

Submitted for publication August 9, 1961

Histology

If there is a diffuse deposition of amyloid the atrial wall is stiff and leathery and the ventricular muscle likewise is firm and rigid. The ventricles do not collapse, but retain a spherical form. Microscopical examination shows the amyloid usually to be deposited in one or more layers in the wall of the arterial blood vessels, especially of the medium-sized and small coronary branches, which may lead to constriction. The muscle fibres are generally surrounded by a network of amyloid, which gives rise to a honeycomb structure. The muscle fibres are under pressure and show atrophy, vacuolization, fragmentation, even necrosis. In the less seriously involved areas parts of the myocardium may be hypertrophic. Not unusually amyloid is also found in the pericardium and the endocardium, resulting sometimes in thickening of the valves. Especially in old age a more focal form of deposition of amyloid like material in the myocardium, probably without much clinical consequences, is quite common (Ho Yong Lee 1957). In these patients with senile focal amyloidosis of the heart little or no amyloid infiltration is found at other sites, while in paramyloidosis of the heart there is usually a considerable amyloid deposition at other sites.

Pathological physiology

Whether amyloidosis of the heart gives rise to circulatory disturbances, and if so, to what degree, depends on the extent of the myocardial infiltration and on the disturbances, if any, of the coronary circulation (amyloid deposition in the vascular wall).

Although amyloid deposition in the pericardium and epicardium is not rare, this does as a rule not impede the cardiac action (Lindsay 1946). This is also true for deposition in the endocardium, unless the valves are also severely infiltrated. This may lead to rigidity resulting into stenosis or incompetence of the valves (Fischer 1958). For example, signs and symptoms of mitral stenosis (Gunnar 1955) and also of stenosis of the aortic, pulmonary and tricuspid valves (Lindsay 1946) have been observed.

More important, however, are — in most of the cases — the sequelae of amyloid deposition in the myocardium. This does not always cause a disturbance of the cardiac function. As mentioned above, in the senile focal amyloidosis of the myocardium there are usually no circulatory disturbances thanks to the limited extent of the infiltration.

If in true paramyloidosis the infiltration in the heart is not severe, signs of cardiac insufficiency may also be absent as was the case in our first patient.

Isolated paramyloidosis of the heart is very rare, usually there is also extensive deposition elsewhere in the body. The pressure on the myocardial fibres leads to serious manifestations of congestive heart failure, to which the changes of the coronary vessels also may contribute. A probably even more important factor is that both the contractile power and the dilatation of the heart are impeded by extensive infiltration of atrial and ventricular walls. Hence the clinical picture may closely simulate that of constrictive pericarditis (Couter et al. 1950, Gunnar et al. 1955) and of endocardial fibro-elastosis (van Buchem et al. 1959). In the first place the heart is usually only little enlarged in comparison with marked congestive heart failure, whilst the excursions of the borders of the heart are only small. If less blood arrives in the pulmonary circulation due to disturbed function of the right ventricle, the lung fields may be clear. However, pulmonary congestion may also occur. The venous pressure is considerably increased, the strongly congested vessels of the neck are remarkable. The liver is greatly enlarged and there is often evident ascites. Many patients show a remarkably extensive oedema, to which may also contribute the usually co-existent hypo-albuminaemia (table I). The pressure curves of the right ventricle, obtained by cardiac catheterization, also show the picture known in constrictive pericarditis (Gunnar 1955) and endocardial fibro-elastosis (van Buchem et al. 1959), viz. an only shortlasting post-systolic fall of pressure ("dip") followed by a rapid rise of the diastolic pressure as a result of the rapid filling of the right ventricle by the high venous pressure and the limited capacity of the ventricle.

The limited contractile power and capacity of the ventricle also becomes usually manifest in the low systolic and pulse pressures and in the low minute volume (Gunnar 1955), which does not increase after loading with albumin infusion (Fischer et al. 1950). Just as in con-



Fig. 1. Case 4. Amyloid infiltration of the mucosa of the lip.



Fig. 2. Case 4. Amyloid infiltration of the enlarged tongue.

strictis pericarditis and endocardial fibroelastosis, these patients react only little to cardiotonics as result of the unfavourable condition of the myocardium.

Clinical manifestations

These have already partially been described. Paramyloidosis especially occurs at advanced age, usually at ages higher than 50 but our patient 1 was only 41 years old. Clinical manifestations are absent if the infiltration of the



Fig. 3. Case 2. Hydrothorax on the right side.

heart is only small. In the case of wider spread the patient often complains of dyspnoea on excertion, while orthopnoea may be absent (slight pulmonary congestion). Often the patient soon notices that he is developing oedema. Complaints of pain in the upper abdomen (especially on the right) with nausea and vomiting and a blown-up feeling in the abdomen as a result of the hepatic congestion are not rare.

More or less typical anginous complaints also occur without overt disturbance of the coronary circulation (Levine 1951). The pulse frequency is usually increased; a pulsus alternans may be present, and arrhythmias, especially atrial fibrillation (our cases 2 and 4) and extrasystoles are not rare. Blood pressure and pulse pressure are usually rather low. The central venous pressure is as a rule markedly increased and is easy to determine in the swollen veins of the neck. The oedema begins in the legs and sacrum and is often extensive, just like ascites and hydrothorax. As in constrictive pericarditis (van Buchem 1957) the hydrothorax is often only unilateral (cases 2 and 3). *The heart is often not very enlarged* (our cases 4 and 5). The heart sounds are often normal, but sometimes

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murmurs, especially systolic, are also audible or there are manifestations of mitral stenosis caused by the rigid mitral valves. The heart has a spherical outline (cases 4 and 5) and kymography often reveals only small excursions. It struck us that some patients showed marked hilar shadows with, perihilarly remarkably wide vascular shadows (our cases 2 4 and 5)

In serious amyloidosis of the heart the ECG is changed, but not pathognomonically Josselson (1953) found no correlation between the severity of the cardiac amyloidosis and the ECG changes. The P and QRS deflections are generally strikingly small in the standard and unipolar leads and sometimes in the precordial leads, the T waves are flat or negative and moreover there is not uncommonly a depression of the ST segment. In the precordial leads I and II the R waves may be absent (our cases 3 and 5) and the ST segments may deviate upwards in an arcuate form, which rises the thought of a myocardial infarction.

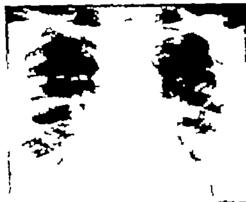


Fig 6. Case 4 Spherical heart

Atrioventricular block and bundle branch block are also observed (Holzmann 1950 Josselson 1953) but more

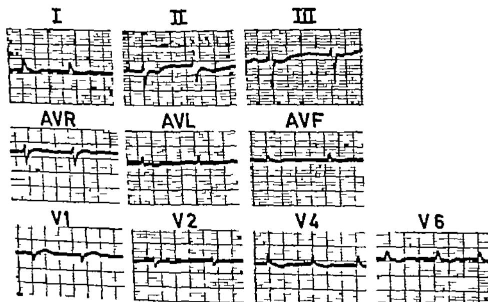


Fig. 7 Case 4 ECG. Auricular fibrillation, low QRS complexes in unipolar and precordial leads. Negative T waves in I, II III V4 and V6.

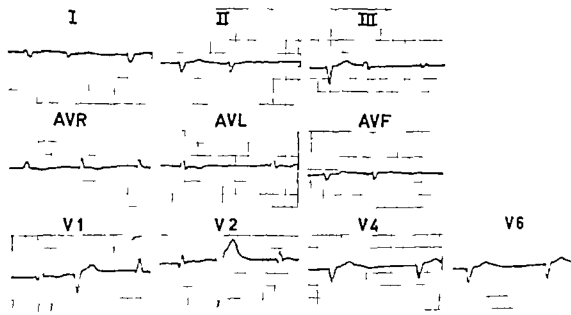


Fig 4 Case 2 ECG Low voltage in QRS and T auricular fibrillation, ventricular extrasystoles.

I II III
AVR AVL AVF
V1 V2 V4 V6

Fig 5. Case 3. ECG Flattened T waves in I AVL, AVF and th precordial leads bsent R wa es in V1 and V2

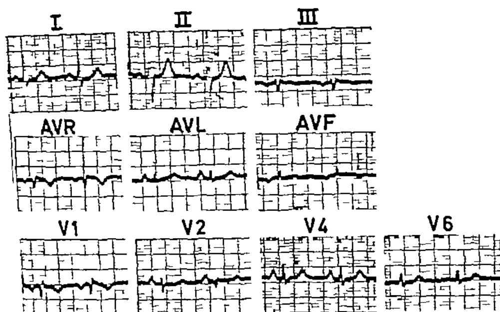


Fig. 10. Case 6. ECG. Low voltage precordial leads.

et al. 1954) Such papulae are not rare either in the mucosae of lip and tongue (figs. 1 and 2) In about 40 of pa tients macroglossia is found due to de position of amyloid in the muscles of the tongue this may be such that the patients have difficulties in eating and speaking this seldom occurs in secondary amyloidosis (Symmers 1956) Deposition of amyloid n the intestinal tract may give rise to diarrhoea, constipation, meteorism pyrosis after meals and haemorrhages. Amyloid infiltration in the larynx may cause hoarseness and dyspnoea. The blood picture usually shows few changes. The number of plasma c lls in two of the five patients in whom the bone marrow was examined, was not learly increased. The ESR may be nor mal or raised (table I)

Analysis of the serum proteins may be of diagnostic significance. Often there is a light hypoproteinaemia with a moderate fall of the albumin and γ-globulin level. On the other hand, there is frequently a relative nd sometimes even an absolute increase of the α_2-globulin and possibly to a lesser degree, of the β-globulin (see table I) Electrophoresis of the serum proteins shows that in a number of patients the α_2 and β-globulins cannot be ery well separated by paper electrophoresis. The "Schlierendiagram remains far removed from the base line. This is sometimes called a curtain effect" It is possible that this is caused by the presence of an extra fraction as described for example, by Block et al. (1956) They called this fraction α 1-globulin. As egard to the paper electrophoresis strips of our patients who showed this so-called curtain effect" between α_2 and β-globulin, we were not able to indicate with certainty the presence of an extra gradient between α_2 and β-globulin. If the strips are tained with Schiff's

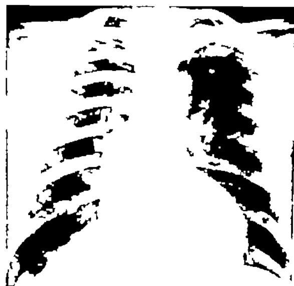


Fig 8. Case 5 Slightly enlarged spherical heart.

often atrial fibrillation Bernreiter (1958) found electric alternation of the P waves.

As shown by figures 4, 5, 7, 9 and 10 the nature of the electrocardiographic changes may be very different, there are by no means always small QRS deflections, even though the amyloidosis is extensive (our case 3).

It is diagnostically important, that not rarely as a result of the paramyloidosis, manifestations may also be found elsewhere in the body.

Goltz reported that in 40 % of 48 patients paramyloid infiltrations were present in skin and mucous membranes (Goltz 1952). Usually these infiltrations are papulae, which, because they are transparent may simulate vesicles, however on palpation they feel solid, their size may range between several millimetres and several centimetres. Their site of predilection is in skin folds, e.g. on the eyelids. Purpura is likewise not infrequent, in which case skin biopsy shows amyloid in the walls of the cutaneous vessels. The purpura is to be explained on the basis of the vascular changes, no disturbance of the coagulation mechanism has been found (Propp

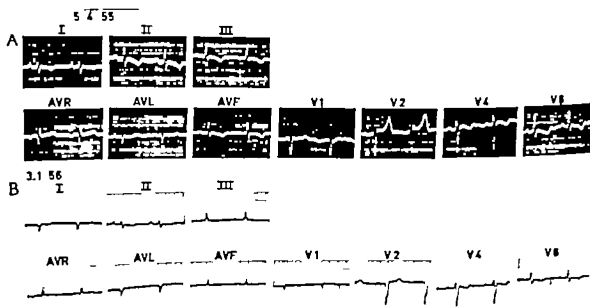


Fig 9 Case 5. ECG A. Negative T waves in I II III AVF V4 and V6, absent R waves in V1 and V2. B. Low voltage QRS complexes with flattened P and T waves in standard and unipolar leads.

whether the amyloid stains metachromatically or not (Ho Yong Lee 1957 Bero 1957) In patients 2 and 3 the localization was characteristic (myocardium tongue, vascular walls) This is probably likewise true for patient 4 who was still alive but in whom amyloid was found in the tongue and the mucous membranes of the lips and in whom the manifestations suggested an extensive amyloidosis of the myocardium. However in all three cases the amyloid showed a clearly metachromatic staining. Patient 5 had, in addition to extensive amyloid deposits in the myocardium, also much amyloid in the liver the spleen and the adrenals. He also showed the clinical manifestations of adrenal insufficiency The amyloid did not stain metachromatically In patient 6 the amyloid deposition in the liver (very large liver) and kidneys was even the predominant feature. The amyloidosis of the liver was diffuse and stained clearly metachromatically The following findings pointed to renal amyloid oedema, marked albuminuria, normal blood pressure, normal renal function, normal intravenous pyelograms, hypalbuminaemia and hypercholesterolaemia (516 mg) This patient showed the same picture as the 24-year old patient of Higgins (1950) (large liver metachromatic staining of the amyloid) who also had a large tongue and in whom amyloid was likewise found in the walls of the small blood vessels.

In patients 5 and 6 with extensive liver amyloidosis, the liver function tests showed the same pattern high cholesterol levels (465 and 518 mg % respectively) high alkaline phosphatase (22.4 and 79.3 King Armstrong units respectively) and normal thymol turbidity test and negative galactose test. In patient 5 the bromsulphalein retention was after 45 minutes 40 % (5 mg/kg) and in patient 6 14 %. It was remarkable that the liver amyloid did not stain metachromatically in patient 5 whereas it did in patient 6 Patient 1 had amyloid deposition in the muscular layer and the muscularis mucosae of the intestinal wall, but not much in the myocardium and the abdominal muscles moreover there was a tumour consisting of amyloid in the second right rib and in the two acetabuli. Biopsy had proved the presence of amyloid in the tongue. There was no amyloid in the liver spleen and kidneys.

With regard to the *differential diagnosis* the affection to be considered first is constrictive pericarditis. As we emphasized before the two clinical pictures are much alike. Several of these patients have therefore been operated upon in the supposition that they suffered from constrictive pericarditis. It is of great importance to search first for changes of skin, mucosae and tongue if there are such changes areas a biopsy can be taken from them. If they are absent, a liver biopsy is to be considered, because amyloid is fairly frequently deposited also in the liver It should however be realized that liver biopsy is somewhat more dangerous in these patients, and indeed has occasionally proved to be fatal (Stauffer) It is therefore important first to try to combat the congestion before liver biopsy is resorted to then Stauffer had no difficulties (Pruitt et al. 1953) In the case of albuminuria, percutaneous renal biopsy is preferable to liver biopsy The chance that the renal biopsy will then be positive is very great even though our cases 1 and 5 are an exception to this rule. It should further be kept in mind that amyloid deposition in the kidney if it accumulates in the marrow may lead to a nephrogenic diabetes insipidus. Deposi-

Table I

Pat No	Sex	Age	Prot. urine (Esbach) ‰	ESR	Bence Jones prot.	Congo red test	Total prot. serum g/100 ml	Alb. %	α %	α_2 %	β %	γ glob. %	Durat. dn.
1	M	41	40	14—34	pos.	neg	4.43	46.3	10.8		[1]29.7	13.2	
2	F	53	neg	11			6.6	59.1	4.5	11.2	11.3	13.9	7 m.
3	F	71	4	54		neg	5.34	36.1	8.4	27.4	13.3	14.6	1¼ y
4	F	71	1	8	neg		6.3	51.2	5.3	13.9	15.7	13.9	>1 y
5	M	58	1.5	6	neg		8.14	44.4	6.2	16.2	14.2	19	
							9.81	46.7	4.8	14.7	11.2	22.6	1¼ y
6	M	59	5—15	47			5.39	39.9	4.7	25.6	17.9	11.9	>1¼ y

[1] $\alpha_2 + \beta$

reagent for the presence of neutral polysaccharides both the α_2 and the β-globulin fractions prove to be rather strongly P.A.S. positive. This increase of hexose bound proteins in the α_2 and β-globulin fractions in amyloid produced experimentally in mice, is a fairly established fact (Catchpole et al. 1958, Mombelloni et al. 1960).

In two of our patients the Congo red test was carried out: the results were negative, but this is also often the case in secondary amyloidosis (Dahlin 1949). Bence Jones protein was only found in patient 1.

The findings in our patients also show that the distinction between paramyloidosis and secondary amyloidosis cannot always be based on the localization (Dahlin 1949, Loogen 1954) or on

Table II

Pat. No.	Liver enlarged	Oedema	Heart enlarged	Murmurs	Circ time	ECG Rhythm	ECG Low volt.	Blood pressure	Venous pres. increased	Amyl. myoc	Weight heart g
1	no	no	norm.	no		sinus	norm.	130/90	no	+	300
2	+	no	+	no	19	aur fibr V. E.	++	125/70 110/65	+	++	585
3	+ ↓ ++	+	±	no	16	sinus	—	130/90	++	+++	545
4	++	+++	+	systol. apex		aur fibr V. E.	++	155/110 100/80	+++	all	
5	++	++	+	no	19	A. E.	— ↓ ++	170/110 110/90	+++	+++	
6	+++	+	norm.	no	15	sinus	+	150/90	+	alive	

The X-rays of the skeleton did not show any changes. Exploratory excision from the tongue paramyloid deposition between the subepithelial connective tissue and between the muscle bundles. Autopsy the bone marrow showed diffuse plasmacytoma tumours in some ribs and the acetabula, consisting of paramyloid accumulations. Paramyloid was, in addition, deposited in muscles, intestinal wall, tongue and heart. Not much paramyloid was found in the myocardium the latter was also fibrotic. Weight of the heart was 300 g. Advanced fatty degeneration of the liver with incipient cirrhosis. The amyloid stained clearly metachromatically with gentian violet.

Case 2. A 53-year-old woman suffered since the past six months, more and more from swollen feet especially at night, and later during the whole day with increasing dyspnoea of effort but no orthopnoea, no palpitations. The abdomen had been swollen since a few weeks before admission, without pain, with a tense feeling. She had been hoarse during the past months due to recurrens paresis. She had suffered from primary chronic rheumatism since thirty years, but the process was inactive since many years.

At admission she had no oedema. The venous pressure was somewhat raised. Blood pressure 110/65. Pulse irregular frequency 82/min. The heart was somewhat enlarged, the sounds were normal. Fluid was present in the right thorax. The liver was somewhat enlarged. Urine analysis was normal, s. g. 1,027 urobilin positive. ESR 11 mm after one hour. Haemoglobin content 12 g erythrocytes 4,000,000, leucocytes 7 700 with normal distribution. Protein spectrum see table I. Wassermann reaction negative. Circulation time (magnesium sulphate) 19 seconds. Chest X-ray fluid in right pleural cavity (fig. 3) the heart did not seem to be much enlarged. Skeletal X-ray did not show any abnormalities. The ECG (fig. 4) showed small deflections of QRS, flat T waves in I, II, AVF negative T in V1 and V2, AVR and AVL, ventricular extrasystoles, auricular fibrillation.

The patient developed epileptiform attacks, from which she died. No bone marrow puncture was carried out. At autopsy an enlarged heart was found (weight 385 g) very firm and elastic to the touch also the atrial wall was thickened. Microscopic examination revealed deposition of paramyloid in the tongue, muscles, vascular walls and myocardium, but not in the liver and brain. Very clear metachromatic staining with gentian violet. No cause of the amyloidosis was found.

Case 3. A 71 year-old woman started with a non-productive cough, some months prior to admission. She also became rapidly dyspnoeic on exertion. During the past few weeks she had had queer feeling in the upper abdomen, but did not vomit. Her appetite was good. On admission she had moderate sacral oedema, no cyanosis. Blood pressure 130/90. Pulse was regular frequency 80/min. Venous pressure was raised. The heart was not enlarged, the sounds normal. Fluid in the right pleural cavity The liver was palpable, first one fingerbreadth, later handbreadth under the costal arch. Urinary findings protein positive, Esbach 4 $^0/_{00}$, urobilin positive, sediment few leucocytes, s. g. 1 028. ESR 54 mm after one hour. Haemoglobin content 10.8 g erythrocytes 4 100,000, leucocytes 5,500 with normal distribution. Cholesterol 271 mg Wassermann negative. Protein spectrum see table I. Congo red test negative. Bone marrow puncture was not carried out.

Chest X-ray much fluid in the right pleural cavity and little on the left, the heart seemed only little enlarged. Circulation time (magnesium sulphate) 16 seconds.

ECG T flat in I AVL, AVF and precordial leads (fig. 5) The manifestations of congestive failure increased gradually She did not react to treatment with strophantin. At autopsy the cut surface of the heart had glassy aspect (weight 545 g) Much amyloid was found in the tongue, the transversely striated muscles and the myocardium, moreover there was amyloidosis of aorta, arteries, arterioles, glomeruli and the vessels of the ovary and the mesenteric vessels. The bone marrow sections did not show any abnormalities. No cause of the amyloidosis was found.

Case 4. A 71 year-old woman suffered since the past year from shortness of breath and easy tiring, especially on some exertion. This had gradually increased. She had already had swollen feet since some years. She had begun to cough only few weeks ago without pro-

tion of paramyloid in the heart as the sole localization of paramyloid although rare, has been observed (Loogen 1954)

The *same differential diagnostic* considerations hold for endocardial fibro-elastosis

It should also be realized that a patient with paramyloidosis may develop dyspnoea *due to amyloid* deposition in the walls of the pulmonary alveoli (Groen 1959) or in the mucosa of the bronchial tree (Mandema 1956) or in the mediastinum Deposition of amyloid *in the branches of the pulmonary artery* may cause increased resistance in the pulmonary circulation with high pressure in pulmonary artery and right ventricle, and finally congestive failure of the *right heart.*

One of our patients (case 2) also had signs and symptoms of rheumatoid arthritis which raised to the question whether the amyloidosis may have been a result *of this disease. In paramyloidosis* muscle and joint complaints occur occasionally usually in the major joints.

The *course of the disease* is very different depending on the progression If manifestations of congestive heart failure have arisen the prognosis is gloomy although it may take many months before the patient succumbs. Although in general the progression of paramyloidosis is slower than of secondary amyloidosis, in the former condition no regression of the process is known, in contrast to secondary amyloidosis, which may *be reversible if* the primary disease has healed.

The *treatment* of these patients can only be palliation for the congestive heart failure. As shown above, not much is to be expected from cardiotonics. Some improvement is possible by prescribing a salt free diet and diuretics. Treatment with liver preparations, ACTH and cortisone has been suggested, but has not been effective (Bero 1957 Parkins et al. 1959) We would not like to recommend ACTH and cortisone, because these hormones proved to promote the experimental amyloidosis obtained with nitrogen mustard (Richter 1954) or with casein injections.

Case reports

Case 1 Some years ago a 41 year-old male developed pains in the chest during work and later more pains in the back and the shoulders. Since a few months he perceived that when he bit his tongue, a blister with blood developed. He had no dyspnoea, swollen feet or nycturia, and he did not cough.

No purpura or oedema were found in the general physical examination The mucosa of tongue and lips showed several papulae filled with blood The edges of the tongue were thickened with formation of papulae Similar glassy looking papulae were present on the inside of the lips and the buccal mucosa at the level of the masticatory surfaces. Blood pressure was 150/90 Pulse regular even, frequency 92/min. Venous pressure was not increased The heart was not enlarged, the sounds normal. Sternum, thoracic vertebrae V and VI and the 12th left rib were painful to pressure. Liver and spleen were not enlarged The urine contained protein (40 /₀₀) and many erythrocytes, s. g 1027 Bence Jones protein positive. Haemoglobin content was *10.3 g %* erythrocytes *3,200,000, leucocytes* 3 400 (eosinophils 2 % staff cells 7 polymorphonuclears 47 % lymphocytes 41 *monocytes* 3) *thrombocytes* 138,000. Wassermann reaction negative. Congo red test negative.

ECG no changes. Many somewhat atypical plasma cells were found in the sternal punctate (57 %) The total protein content of the serum was decreased (4 43 g) The protein spectrum showed a lowered albumin content (46 3) and relatively increased $\alpha_2 + \beta$-globulin content (29 7) See table I

The $\alpha_2 + \beta$-globulin content was also raised in absolute figures. There was no good separation between the α and β-gradient, so that these could not be determined separately

On admission he had pretibial and sacral oedema, increased venous pressure, blood pressure 150/90, pulse regular frequency 90/min. The heart was not enlarged, the sounds were normal. The lungs were emphysematous, the liver firm and markedly enlarged, the spleen was not palpable. The urine contained much protein (5—15 /₀₀), the urobilin reaction was strongly positive, s. g. 1,019 sediment few leucocytes. Urea clearance 74 %, phenolsulphonphthalein test 74 %. Serum bilirubin 0.7 units, alkaline phosphatase 29.3 King Armstrong units, thymol turbidity test 0.4 units, cholesterol 518 mg %, cholesterol esters 423 mg %, bromsulphalein retention after 45 minutes 14 % (5 mg/kg) galactose test, no excretion; S.G.O.T. 33 units. ESR 47 mm after one hour. Haemoglobin 12.4 g %, erythrocytes 4,000,000, leucocytes 9,100 with normal distribution. Wassermann negative. Circulation time 15 seconds (magnesium sulphate). Chest X ray low pulmonary borders, heart not enlarged. ECG low voltage of precordial leads (fig. 10). Many small plasma cells in the sternal marrow.

Liver biopsy: diffuse deposition of amyloid. Gingival biopsy: no amyloid. Neither in the teeth nor in the throat, mouth or nose was a purulent process found. Nor was a cause for the amyloidosis found elsewhere in the body.

Summary

The authors observed six patients with paramyloidosis. In four of them the sequelae of an extensive cardiac amyloidosis were the main feature. The pathological physiology and the signs and symptoms of cardiac amyloidosis are discussed. During life the diagnosis can usually be made on the amyloid depositions that generally occur also at other sites of the body (skin, mucous membranes of the mouth, tongue, liver, kidney). The localization and the metachromatic staining of the amyloid had only limited value for the differentiation of para-amyloidosis and secondary amyloidosis.

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Biopsy of the infiltration of the lip: much amyloid; gingival biopsy: some amyloid deposition. The patient responded poorly to therapy with cardiotonica. No cause of the amyloidosis was found.

Case 5. A 58-year-old male tired easily since several years and suffered from heartburn and nausea. Since some months his legs became swollen, he became short of breath on exertion, and he had palpitations. He coughed little and did not produce sputum. The legs became thin again after due rest. On a few occasions he had had a rather severe nose bleeding. On admission he had no oedema.

The jugular veins were congested, the venous pressure was markedly increased. Blood pressure 170/110, later 110/90. Pulse irregular due to extrasystoles, frequency 98/min. The heart was moderately enlarged, the heart sounds pure. The liver was palpable a handbreadth under the costal arch. The spleen reached two fingerbreadths under the costal arch. The urine contained 1.5 ‰ protein. Bence Jones protein negative, urobilin positive, s. g. 1.024. Urea clearance 99 %. Haemoglobin 14.4 g %, erythrocytes 4,900,000, leucocytes 11,000 with normal distribution. ESR after one hour 6 mm. Wassermann reaction negative. Some liver function tests were disturbed (bromsulphalein retention after 45 minutes 90 %, bilirubin indirect 1.7 units, later direct positive 6.5 units, thymol turbidity test 1.3 units, alkaline phosphatase 16.9 King Armstrong units). Protein spectrum: see table I. Circulation time: 19 seconds (magnesium sulphate).

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Liver biopsy: a diffuse pink homogenous substance was found between the atrophic liver trabeculae; this substance greatly resembled amyloid. The amyloid staining was negative, probably paramyloid. Skull photo: translucent areas reminiscent of myeloma, but not convincing.

Sternal marrow biopsy: increased number of small plasma cells (14 %). The patient gradually deteriorated. The skin showed brown pigmentations. The patient was very tired. The blood pressure fell to 80/60. After injection of 25 mg ACTH the eosinophil leucocytes fell from 32 × 3 to 20 × 3, and on repetition there was no fall at all. Serum sodium 138 mEq, serum potassium 5.8 mEq. There was therefore an evident adrenal insufficiency. Autopsy: much amyloid was deposited in liver, spleen, adrenals and especially in the heart. The bone marrow also contained amyloid, but the kidneys did not. No cause of the amyloidosis was found.

Case 6. A 59-year-old man had suffered from bronchial asthma for many years already. Of late he had swollen ankles and legs, nycturia, and he became rapidly short of breath on exertion. He easily developed a full feeling in the gastric region; defaecation was normal.

On admission he had pretibial and sacral oedema, increased venous pressure, blood pressure 150/90, pulse regular frequency 90/min. The heart was not enlarged, the sounds were normal. The lungs were emphysematous, the liver firm and markedly enlarged, the spleen was not palpable. The urine contained much protein (3—15 ‰), the urobilin reaction was strongly positive, s. g. 1,019 sediment few leucocytes. Urea clearance 74 %, phenolsulphonphthalein test 74 %. Serum bilirubin 0.7 units, alkaline phosphatase 29.3 King Armstrong units, thymol turbidity test 0.4 units, cholesterol 318 mg %, cholesterol esters 423 mg %, bromsulphalein retention after 45 minutes 14 % (5 mg/kg) glucose test no excretion S.G.O.T 33 units ESR 47 mm after one hour Haemoglobin 13.4 g % erythrocytes 4,000,000, leucocytes 9,100 with normal distribution. Wassermann negative. Circulation time 15 seconds (magnesium sulphate) Chest X-ray: low pulmonary borders, heart not enlarged. ECG low voltage of precordial leads (fig 10). Many small plasma cells in the sternal marrow

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Incidence of Gastric Cancer in Patients Treated in Medical Services for Gastric Ulcer

By

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For more than one hundred years the question of a relationship if any of gastric ulcer to gastric cancer has been of the greatest concern to clinicians and pathologists. In 1842 Rokitansky (14) found that the two lesions might develop concurrently he advanced the theory that cancer might arise from apparently benign ulcers which theory still is prevalent and in particular considered correct by surgeons in our time. In further support of this contention Newcomb (11) found it possible to decide on the basis of histological examination whether cases of gastric cancer originated from ulcerations, always provided that the lesion was not too far advanced Another theory was set up in 1898 by Duplant (5) who maintained that the finding of cancer cells in gastric ulcers suggested that cancer represented the primary disease the diagnosis of benign ulcer merely being an incorrect clinical diagnosis not to be realized until n later stages. At a comprehensive conference held in Columbia in 1952 it was concluded — in conformity with Duplant's theory that a development of cancer in preexistent gastric ulcers was of questionable occurrence and never verified.

In clinical routine the problem is confined to ascertain whether an existing gastric ulcer is of a benign or malignant nature, viz. whether the clinical diagnosis is considered sufficiently reliable to allow for a conservative treatment of the disease which, when benign generally responds well to cure, or whether surgical measures should be taken immediately always considering the uncertainty of diagnoses and the suspicion of malignancy It has been maintained by surgeons that the differential diagnoses may be rather uncertain and that not infrequently gastric cancers are treated in medical services the lesion being misdiagnosed as gastric ulcer Hence we have considered t of interest to review the frequency of occurrence of gastric cancer in patients in whom the presence of a gastric ulcer has been radio-

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problem of gastric ulcers treated in medical services was published by Krogsgaard (9). Among 84 patients treated by stomach tube in whom the presence of gastric ulcer had been verified by radiological examination this author found 2 cases of gastric cancer (2.4 per cent), patients being followed for 3 1/2 years; three patients had been lost sight of. In our material are included some of Krogsgaard's cases, these patients having been in the department after 1945.

Table I. Distribution of the 284 patients excluded from the originally registered 532 cases of ulcus corporis ventriculi, prepyloric ulcer, haematemesis and melaena

No radiological examination	56
Radiological findings normal	153
Radiological findings uncertain	69
Deaths during hospitalization	6
Total	284

Material and technique

Requirements for the material have included: clinical diagnosis of gastric ulcer at discharge from the department, this diagnosis being established on the basis of radiological findings of niches on the lesser curvature; follow-up periods covering at least 5 years; and a high percentage of patients appearing for control at the time of review.

The material consists of patients admitted to Department C, Bispebjerg Hospital, Copenhagen, between January 1 1945 and December 31 1954. During this period 532 patients were filed in the in-patients' record office of the department under the diagnoses ulcus corporis ventriculi, prepyloric ulcer, haematemesis, and melaena. Patients were selected in whom niches were demonstrated beyond doubt on the lesser curvature (206 cases) or to whom surgical measures had been applied immediately before transfer to the medical department, operation having been dictated by perforation of the ulcer (42 cases). All surgical measures applied have included suture of perforation. Records from the surgical department have been reviewed in order to ensure that patients are excluded in whom ulcers were localized on the duodenum. Prepyloric ulcers in particular are found to be prone to malignancy and consequently patients presenting this lesion have been included here although the radiographical differentiation of duodenal and prepyloric ulcer is known to be rather uncertain; surgical measures at later stages in 73 cases gave rise to some doubt in 9 of the cases, viz. whether the lesions were of prepyloric or duodenal character.

Of the 532 originally registered patients 284 were excluded because requirements to our criteria were not fulfilled, viz. the radiological demonstration beyond doubt of a niche on the lesser curvature or the finding at operation of an ulcer. These 284 patients are classified in table I.

Indeed, such selection may involve that certain patients are excluded in whom gastric cancer either might have been present or may have developed at later stage but, it has been our express desire to provide a distinctly outlined standard of the material.

Thus the material finally includes 248 patients who have been traced either with the assistance of the National Registration Office or by other means. At the time of review 197 of these patients were still alive; with one exception they have all been contacted. The exception was an alien citizen who during a sojourn in Denmark was treated for gastric ulcer. All the other patients have answered a questionnaire requiring information of surgical treatments, if any, after discharge from this department. The data of operations and histological diagnoses, if established, have been obtained by inquiries to the surgical departments concerned or by review of the surgical records. Death certificates or autopsy records, if available, have served as means of information if patients had died. From relatives of non-autopsied patients information has been obtained as regards the assumed surgical measures. This information has been controlled like the rest of such treatments. Thus the percentage of patients of whom information has been obtained at the time of review ranges at 99.6.

logically verified and consequently treated in a medical service all cases have been followed for adequate periods. It is not the intention here to weigh against each other the benefits of medical and surgical treatments of gastric dyspepsia modern medical as well as surgical publications have discussed this problem in detail

As was mentioned above the differential diagnoses have been of great concern to numerous investigators and the literature contains hundreds of reports discussing the problem of gastric ulcer versus gastric cancer Criteria are manifold on which investigations have been based. Statements of percentage at which cancer develops in lesions clinically diagnosed as ulcers vary from 0 to 90

The present paper is concerned only with modern studies, particularly Scandinavian.

On the basis of personal, histological examination Newcomb (11) found malignancy in almost 4 per cent of all gastric ulcers, and states also that 13 per cent of all cases of gastric cancer develop in apparently benign ulcers. Stewart (17) examined 1,503 resected stomachs and found that 10 per cent of the gastric ulcerations were of malignant nature, almost 18 per cent of all gastric carcinomas had developed in chronic benign ulcers Wulff (19) examined 609 cases of gastric cancer this author found it justifiable to suggest that 8 per cent of these were encountered in patients with a previous history of gastric ulcer Dahl Iversen (3) reported 11 cases (12 per cent) of carcinoma in 94 patients subjected to operation for gastric ulcer Among 485 surgical cases of gastric cancer Koster (10) found a previous history of gastric ulcer in 20 per cent.

Smith Boles & Jordan (16) found 7 carcinomas in 422 cases of gastric ulcer (less than 2 per cent) treated in medical services The cause of death was unknown in 5 cases and the briefest follow-up period covered two months only Of 908 conservatively treated patients Bille & Romcke (2) found 26 cases of cancer (3 per cent) the patients were followed for from 6 months up to 14 years the number of patients who were lost sight of is not recorded Doll Avery Jones, Pygott & Stubbe (4) followed for three years 285 patients who were divided into two categories viz. those in whom the clinical diagnosis of benign ulcer had been established beyond doubt (266 cases) and those in whom malignancy was suspected (19 cases) these authors found cancer development in one patient in the large group (0 4 per cent) and in four patients in the small group (21 per cent) Two comprehensive Danish medical reports are available. Åge Nielsen (12) reported 12 cases of gastric cancer in a material including 324 patients treated in medical services and followed for at least 2 1/2 years no differentiation has been made in this material between gastric ulcer and duodenal ulcer Krarup (8) reviewed 665 cases of peptic ulcer followed for at least 5 years. 81 patients had gastric ulcers (demonstration beyond doubt of a niche on the lesser curvature) In the other cases the site of the ulceration was either obscure or the patients were suffering from duodenal ulcers. 6 cases of gastric cancer were found In addition to these two studies Seedorff (15) has reported 78 cases admitted to medical departments and found development of gastric cancer in 5 per cent the patients being followed for 5 years statements of causes of deaths are rather indeterminate as regards 10 additional patients, e g cachexia and melaena. However the most reliable Danish review with relevancy to our

problem of gastric ulcers treated in medical services was published by Krogsgaard (9). Among 84 patients treated by stomach tube in whom the presence of gastric ulcer had been verified by radiological examination this author found 2 cases of gastric cancer (2 4 per cent) patients being followed for 3 1/2 years three patients had been lost sight of. In our material are included some of Krogsgaard's cases, these patients having been in the department after 1945.

Material and technique

Requirements for the material have included clinical diagnosis of gastric ulcer at discharge from the department, this diagnosis being established on the basis of radiological findings of niches on the lesser curvature follow-up periods covering at least 5 years and high percentage of patients appearing for control at the time of review

The material consists of patients admitted to Department C, Bispebjerg Hospital, Copenhagen, between January 1 1945 and December 31 1954. During this period 532 patients were filed in the in-patients record office of the department under the diagnoses ulcus corporis ventriculi, prepyloric ulcer haematemesis, and melaena. Patients were selected in whom niches were demonstrated beyond doubt on the lesser curvature (206 cases) or to whom surgical measures had been applied immediately before transfer to the medical department, operation having been dictated by perforation of the ulcer (42 cases) All surgical measures applied have included suture of perforation. Records from the surgical department have been reviewed in order to ensure that patients are excluded in whom ulcers were localized on the duodenum. Prepyloric ulcers in particular are found to be prone to malignancy and consequently patients presenting this lesion have been included here although the radiographical differentiation of duodenal and prepyloric ulcer is known to be rather uncertain surgical measures at later stages in 73 cases gave rise to some doubt in 9 of the cases, viz. whether the lesions were of a prepyloric or duodenal character

Table I Distribution of the 284 patients excluded from the originally registered 532 cases of ulcus corporis ventriculi, prepyloric ulcer haematemesis and melaena

No radiological examination	56
Radiological findings normal	153
Radiological findings uncertain	69
Deaths during hospitalization	6
	Total 284

Of the 532 originally registered patients 284 were excluded because requirements to our criteria were not fulfilled, viz. the radiological demonstration beyond doubt of a niche on the lesser curvature or the finding at operation of an ulcer These 284 patients are classified in table I.

Indeed, such selection may involve that certain patients are excluded in whom gastric cancer either might have been present or may have developed at a later stage but, it has been our express desire to provide a distinctly outlined standard of the material.

Thus the material finally includes 248 patients who have been traced either with the assistance of the National Registration Office or by other means. At the time of review 197 of these patients were still alive with one exception they have all been contacted. The exception was an alien citizen who during sojourn in Denmark was treated for gastric ulcer All the other patients have answered questionnaire requiring information of surgical treatments, if any after discharge from this department. The data of operations and histological diagnoses, if established, have been obtained by inquiries to the surgical departments concerned or by review of the surgical records. Death certificates or autopsy records, if available, have served as means of information if patients have died. From relatives of non-autopsied patients information has been obtained as regards the assumed surgical measures. This information has been controlled like the rest of such treatments. Thus the percentage of patients of whom information has been obtained at the time of review ranges at 99.6.

Table II Classification of patients according to age and sex

Age	Men	Women
< 30	3	4
30—39	19	9
40—49	46	24
50—59	55	23
60—69	35	15
70—79	10	4
> 79	1	0
Total	169	79

Table IV Distribution of patients according to duration of follow-up and fate

Follow-up Years	Alive at time of review		Deaths	Unknown	Total
	Non resected	Resected			
5— 7	52	23	14	1	90
7—10	46	30	17	0	93
10—15	25	20	20	0	63
Total	123	73	51	1	248

Results

Classification according to age and sex is shown in table II The percentage distribution is registered in table III in which Krarup s material from 1946 and Krogsgaard s from 1953 serve as standard of comparison.

Whereas the incidence is equally high in men and women in Krarup s (8) material Krogsgaard s and our material present twice as many male as female cases. This is in conformity with Alsted s (1) and Hansen s (7) findings viz. that the disease has changed from being a disorder most frequently encountered in female patients to be one mostly occurring in male patients. The age distribution in the three materials is also striking In Krarup s material incidence is highest in men at ages between 30 and 40 years in Krogsgaard s material incidence is highest in patients at ages between 40 and 50 years whereas incidence in our material is highest in men at ages between 50 and 60 years. No such difference is noted in the female cases of the three materials including patients admitted to Bispebjerg Hospital between 1930 and 1945.

In table IV patients are classified according to duration of follow-ups and as to fate.

Table III Distribution in per cent of patients according to age and sex collated with Krarup s material from 1946 and Krogsgaard's from 1953 The three groups of patients have all been treated in the Bispebjerg hospital in the period from 1930 to 1945

	Age < 30 M	< 30 F	30—39 M	30—39 F	40—49 M	40—49 F	50—59 M	50—59 F	60—69 M	60—69 F	70—79 M	70—79 F	> 79 M	> 79 F	Number M	Number F
	%															
Krarup (1946)	18	5	36	7	31	21	5	26	10	26	0	12	0	2	39	42
Krogsgaard (1953)	3	16	18	10	40	23	25	26	12	19	2	6	0	0	57	27
Lyngborg & Nielsen (1961)	2	5	11	11	27	30	33	29	21	19	6	5	(0.5)	0	169	79

Table V Causes of death of 21 non-autopsied patients out of the 51 who died during the follow-up period

Coronary arterial occlusion	3
Mitral stenosis	1
Non-specified cardiac disorders	3
Arteriosclerotic gangrene	1
Cerebral apoplexy	2
Cancer pulm. bronch..	2
Carbonmonoxide poisoning	1
Gastro-intestinal or hepatic disorders	8
Total	21

Table VI Causes of death in the group "gastro-intestinal and hepatic disorders" recorded in table V

Gastric cancer	4
Anuria after partial gastrectomy	1
Hepatic cirrhosis	1
Pulmonary tuberculosis, abdominal tumour	1
Cerebral arteriosclerosis abdominal tumour	1
Total	8

Of 73 patients subjected to surgical treatment and still alive at the time of review 25 were transferred directly to the surgical department 20 patients were subjected to operation within the ensuing year the a erage interval to operation of the remaining 28 patients was 4 years. In 30 utopsied patients evidence of partial gastrectomy was noted in 11 cases. In 21 non-autopsied patients the cor responding figure was 4 Hence 88 of the 247 patients, the fates of whom are known, have been subjected to surgical treat ment. The excised stomach specimens have been histologically examined in 69 of the cases. Evidence of malignancy was noted only in one case, the diagnosis of incipient adenocarcinoma having been established on the basis of findings of atypical, proliferating nodules. These preserved slides ha e been evised at the Pathological Institute of the Bispebjerg Hospital (Steen Olsen) but neither typical areas nor evidence of malignancy could be found. In four cases there had been no histological examination of the excised specimens.

In the group including the 51 deceased patients 30 wer utopsied and gastric cancer demonstrated in 3 cases. The causes of death of the 21 non-autopsied patients are recorded in table V

The category gastro-intestinal and hepatic disorders" is classified in table VI

In four non-autopsied cases the cause of death was gastric cancer One patient who died in anuria had been subjected to partial gastrectomy immediately before death occurred the histological examination of the stomach specimen gave no evidence of malignancy One patient presenting symptoms of cirrhosis had been in the medical department until shortly before death. One patient who died from pulmonary tuberculosis had been subjected to partial gastrectomy 5 years previously at which time the excised specimen presented no signs of malignancy The patient died in a sanatorium and physicians had diagnosed n enlargement of the liver and tumour development in the right iliac fossa. Finally one patient belonging to this category died t the age of 83 One year before death he had been in a psychiatric department with senile dementia there is no record from this department showing a history of gastric dyspepsia during hospitalization the haemoglobin had been 90 per cent, the sedimentation rate 33 mm. The physician drawing up the death certificate did not know why a diagnosis of gastric cancer has been established

Table VII Survey on the 7 cases of gastric cancer encountered in the present material

No.	Admission	Sex	Age at admission	Duration of previous history	Gastric acid test	No. of X ray examinations	Site of lesion	Interval to death
1	1946	M	53	3 months	— acid	2	Fundus corpus	5 months
2	1948	M	68	6 months	?	0	Lesser curvature	10 months
3	1951	M	45	1 month	+ acid	1	Lesser curvature	3.5 years
4	1952	M	68	10 years	+ acid	1	Lesser curvature	5 years
5	1953	F	65	6 months	?	2	Fundus corpus	13 months
6	1953	F	53	?	?	1	Lesser curvature	3 months
7	1955	M	82	3 months	?	1	Corpus	2 years

Five patients died immediately after the performance of partial gastrectomy one of these operations being performed on the indication of gastric cancer During the period of follow up one patient only has died from haemorrhage (haematemesis) no deaths have occurred due to gastric perforation

The 7 cases of gastric cancer are recorded in *table VII*

Case reports

Case 1 Man aged 53 for three months complaining of hunger pains. No loss of weight. Haemoglobin 94 to 106 per cent sedimentation rate 20 to 7 mm. Ewald's test meal absence of free acid. The radiological examination showed a niche on the lesser curvature at the junction of the fundus, a suspected ulcer A control 20 days later showed some enlargement of the niche. After a 41 day-ulcer-diet the patient was discharged the diagnosis being established as a suspected gastric ulcer suspected gastric cancer 45 days later the patient was admitted to another department where the radiological examination showed the development of gastric cancer Biopsy from nodules on the neck showed the presence *of a solid scirrhous* carcinoma.

Case 2 Man, aged 68, transferred from the surgical department to have an ulcer diet after an operation for suture of a perforated ulcer For 6 months the patient had been complaining of oppressing epigastric pains. Operation had revealed a tumour type involvement of the greater part of the lesser curvature. Biopsy during operation disclosed acute and chronic inflammatory changes but gave no evidence of malignancy Two days after transfer to the medical department the patient insisted on being discharged. After an interval of three months the patient was readmitted presenting symptoms of pylorostenosis he was transferred *to the surgical* department where operation disclosed a metastasizing gastric cancer

Case 3 Man, aged 45, admitted on account of haematemesis, presenting a previous history of ulcer type pains of one month's duration. Haemoglobin 55 to 88 per cent sedimentation rate 7 mm. Benzidine reaction from +++ to — Radiological examination showed an ulcer on the lesser curvature The radiologists recommended to have a control film but it was never carried out. Ewald's test meal normal contents of free acid. The patient was discharged relieved of his symptoms he felt well until three years later when he was re admitted complaining of uncharacteristic dyspeptic pains lasting for one month. Repeated radiological examination gave rise to a suspicion of cancer and the patient was transferred to the surgical department for operation which disclosed an inoperable gastric cancer

Case 4 Man aged 68 suffering from melaena at admission For several years he had been complaining of ulcer type dyspeptic pains. Haemoglobin 48 *to* 69 per cent sedi-

mentation rate 3. to 80 mm. Benzidine reaction from +++ to — Ewald' test meal: normal contents of free acid. The radiological examination showed an ulcer on the lesser curvature. At discharge the patient felt well. The main diagnosis was established as: ulcer on the lesser curvature with the additional diagnosis of suspected tumour of the stomach, although it is not apparent from the record why this sub-diagnosis had been made. 5 years later the patient was re-admitted (aged 73). For the previous 6 months the patient had been complaining of epigastric pains he had lost 20 kg. The patient was rather cachectic: the benzidine reaction remained positive: the sedimentation rate was 150 mm. A few weeks later the patient was discharged the diagnosis being established as suspected gastric cancer: cerebral arteriosclerosis. Tw days later he died at home. Although there was no autopsy the diagnosis of gastric cancer can hardly be doubted.

Case 5 Woman, aged 65, who previously had been operated upon for mammary cancer. One year before the present admission indicated by her dyspeptic pains she had been treated for left-sided recurrent paresis the origin of which was never discovered. On admission the patient was complaining of epigastric pains which had been persisting for 6 months. She had lost 10 kg. An epigastric tumour was palpable. Haemoglobin 100 per cent; sedimentation rate 4 mm the benzidine reaction was negative on 6 occasions. Gastric juice was not examined. The radiological examination showed duodenal ulcer and certain irregularities in the gastric fundus hich, by repeated radiological examination, were diagnosed as ulcerations t the junction of the corpus ventriculi and the fundus. 18 days later the patient was discharged the diagnosis being established as gastric ulcer suspected gastric cancer: it was added that "since the patient feels well we btain from any further examination of the suspected cancer 13 months later the patient died. A large gastric cancer with involvement of the pleura and pericardium was found at autopsy

Case 6 Woman, aged 53 transferred from the psychiatric department on account of haematemesis. The previous history was rather confused because of the mental state of the patient. On admission omiting was continuous. Haemoglobin 72 per cent sedimentation rate 34 to 46 mm the benzidine traction was positive on all occasions. Radiological examination showed an ulcer on the lesser curvature. The radiologists suggested that the lesion might be a neoplasm. Gastric juice was not examined. As vomiting persisted the patient was transferred to the surgical department for operation of the gastric ulcer Histological examination of the excised stomach gave evidence of gastric cancer the patient died on account of inadequate sutur ing: nodular metastases were found at autopsy

Case 7 Man aged 82 suffering from melaena. Three months before admission the patient had been in the department, also because of melaena, but at that time the radiological examination had failed to exhibit any gastric anomalies. Radiological examination was repeated on the second admission and showed an ulcer on the lesser curvature. During hospitalization the benzidine reaction remained negative. Gastric juice was not examined. The patient was discharged one month later the diagnosis being established as ulcus corporis ventriculi. After an interval of two years the patient was re-admitted. He had lost 30 kg and died in hospital. A large ulcer on the lesser curvature perforating into the lesser peritoneal sac was found at autopsy The death certificate bore the legend ulcus corporis ventriculi but the histological examination gave evidence of an adenocarcinoma.

Discussion

In the present material including 248 patients confidently diagnosed as having gastric ulcers the development of gastric cancer has been unambiguously demonstrated in 7 cases, the patients being followed for at least 5 years. Death certificates of 2 additional, non-autopsied patients are diagnosed as abdominal tumour hence the theory cannot be dismissed that these patients have died from gastric cancer Thus the percentage of cancer development in the present ma-

Table VIII Incidence of cancer in various groups of patients admitted to medical services on diagnosis of gastric ulcer

Authors	Year of publication	No. of patients	Cases of cancer	Traced patients	Briefest follow-up	Cancer development in
Bille & Romcke	1948	908	26	?	6 months	2.9
Swynnerton & Tanner	1953	254	6	94.5	6 years	2.4
Smith, Boles & Jordan	1953	422	7	?	2 months	1.2
Krogsgaard	19 3	84	2	96.4	3.5 years	2.4
Doll, Avery-Jones et al.	1957	307	5	96.8	3 years	1.6
Lyngborg & Nielsen	1961	248	7	99.6	5 years	2.8

terial has been found to be 2.8 and 3 6 respectively The entire material consisted of 159 patients who were not subjected to operation A calculation of the percentage at which cancer develops in this group gives the figure of 4 4 (5 0 if the "abdominal tumour be considered as gastric cancer partial gastrectomy had been performed on one of the two patients to whom this diagnosis had been fixed)

Incidences of cancer development in the present material and in a material consisting of ordinary subjects are compared age and sizes of the two groups being identical [1] The incidence of gastric cancer is found to be 4 to 5 times higher in our patients with gastric ulcer than in the group of ordinary subjects. However the small size of the material should be borne in mind and the difference demonstrated here may be fortuitous.

Results are recorded in table VIII where they are compared with findings from other studies on the incidence of cancer development in patients submitted to treatment for gastric ulcer

[1] Calculations are made by M Svend Sorensen, Actuary the Danish Cancer Register Dr Johannes Clemmesen, M. D Head of the Cancer Register has most kindly placed the figures at our disposal.

The table shows that the incidence of cancer development in the various materials is almost of the same order When surgeons have maintained that the incidence of cancer is at a higher level than here stated, as e g in Danish surgical reports it should be remembered that such surgical materials are selected materials. This feature is clearly demonstrated by the investigation carried out by Smith Boles, & Jordan (15) who reexamined 1 000 cases diagnosed as manifestations of gastric ulcers (peptic ulcers) the authors have paid no regard to the factors benignancy and malignancy before examination 578 patients were subjected to operation and cancer was diagnosed in 81 of these. 422 patients were not subjected to operation and gastric cancer was seen in 7 of these. A calculation of the incidence of cancer development in the entire material gave a figure of 8 8 per cent The percentage of cancer development in patients subjected to operation was found to be 14 And cancer was seen to develop in 1 7 per cent of the patients with gastric ulcer who were conservatively treated Thus it will be noted that surgically treated patients represent a selected material which feature also is apparent when it is realized

that a suspicion of malignancy dictated the performance of operations in 40 per cent of the cases. Consequently the incidence of cancer development in patients subjected to gastric operation cannot be compared with incidence in patients with gastric ulcers. The number of patients who before operation had been on gastric ulcer diets provides no indication as regards a cancer development from gastric ulcer because such diets are not infrequently merely administered as a means of observation until the time when a suspected malignancy of the case calls for operation, e g falling response to the conservative treatment.

However it should be noted that the rate of mortality from gastric cancer seems to be particularly high among patients with gastric ulcers although the present material must be admitted to be too small to allow for a reliable statistical evaluation to be made.

In estimating the hazards of cancer in patients treated in medical services for gastric ulcers the post-operative mortality of operated patients should be borne in mind. In the present material mortality among such patients is about 5 per cent which figure is definitely higher than the figure of cancer development even if all uncertain diagnoses be considered as cases of gastric cancer

In the large, modern, surgical departments the mortality among gastrectomized patients range about 2 per cent only (Fishermann & Rasmussen (6) but diagnostic appliances have much improved since the time ten years ago when the major part of the present material was seen. According to modern standards most of these older cases admittedly were inadequately examined presenting symptoms which, in retrospect, should have provided sufficient indication for performance of operation. It is hardly possible to establish a differential diagnosis which is 100 per cent correct, but careful examination of the patient, including benzidine reactions, examinations of gastric juice cytological examination (exfoliative gastric cytology) repeated radiological control, gastroscopy and the institution of surgical treatment of all doubtful cases, should certainly bring hazards of misdiagnosis below the level, or on the level of the post-operative mortality

Summary

The incidence of gastric cancer is examined in 248 patients (169 men and 79 women) admitted to a medical department during the period from 1945 to 1955 under the diagnosis of gastric ulcer diagnoses being established on the basis of radiological findings of niches on the lesser curvatures (206 cases) or the finding at laparotomy of perforating ulcers of lesions on the corpus ventriculi (42 cases) Patients have been followed for at least 5 years. Information of the fates of these patients has been obtainable in 99 6 per cent.

At the time of review 197 of the patients were still alive 51 of the patients had died. Partial gastrectomy had been performed in 73 of the former cases. The resected specimens had been histologically examined in 69 of the cases, none of which bore evidence of malignancy

Of the 51 deceased patients 30 were autopsied and a development of gastric cancer was found in 3 of these In 21 non-autopsied cases cancer represented the cause of death in 4 cases to these should be added 2 more patients in whom the diagnosis of 'abdominal tumour' had been established naturally there is no evidence to the effect that the cause of death was actually gastric cancer

Whether or not we include these latter two cases in calculations of the percentage of cancer development noted at the review of the entire material, figures will be 2 8 or 3 6 159 of the patients were not subjected to operation In this group development of cancer was seen in 4 4 per cent of the cases (5.5)

The incidence of cancer in the present material is 4 to 5 times higher than the *incidence of gastric cancer in a group of* non selected subjects.

The mortality of gastrectomised patients in the present material was 5 per cent The operative mortality has since decreased but diagnostic procedure has much improved in the same time.

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The Effect of an Anabolic Steroid, Nor-Testosterone Phenyl Propionate (Durabolin®), on the Urinary and Plasma Steroids in Patients with Normal and Decreased Renal Function

By

E. Ihalo A. Kasanen N. Kärki and E. Tala

The use of testosterone in the treatment of patients with kidney disease is understandable, because androgenic hormones cause a positive nitrogen balance, inhibiting the breakdown of protein (9 12, 23). It has been shown too, that test animals survive longer after bilateral nephrectomy when given testosterone (10). Testosterone has an especially beneficial effect on the nephrotic type of nephritis, e. g. proteinuria decreases and the non-protein nitrogen (N. P. N.) drops while the total plasma proteins rise in rats (13).

New anabolic hormones have a greater protein-conserving than virilising effect. When e. g. Nilevair® (17-ethylnortestosterone) was administered to obstetric patients with acute renal failure a 70 per cent decrease occurred in the protein catabolism from the pre-therapeutic level (11). Gjørup and Thaysen (5) also treated acute renal failure with anabolic steroids. This treatment, which conserves body proteins, is of value in preventing the occurrence of late complications of acute renal failure and shortens the period of convalescence (1).

Several investigations suggest that the basal excretion of 17-ketosteroids (17 KS) and 17 hydroxycorticosteroids (17 OHCS) in the urine in renal insufficiency is often reduced thus pointing to the possibility of steroid retention. Retention of 17-OHCS in the plasma and especially that of conjugated 17-OHCS seems to correlate with the degree of renal damage. This retention is most pronounced in the evening (3, 8, 15, 17, 22). Elevated 17-OHCS values in these patients can sometimes be as high as the findings established in Cushing syndrome.

In renal insufficiency the basal excretion of 17 KS in the urine is reduced. When testosterone was given intramuscularly 100 mg daily for five days, the total 17 KS excretion was clearly lower 69.4 mg in severe renal insuffi-

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Table I. The effect of an anabolic steroid, Durabolin (nor-testosterone propionate) on the urinary excretion of 17-hydroxycortico- 17-keto- and 17-ketogenic steroids and 17-hydroxycorticosteroid content in the plasma of patients with normal and decreased renal function

Group	Plasma level µg/cm³ + S. E. M.				Excretion in urine mg/day + S. E. M.					
	Free 17-OHCS		Conj. 17-OHCS		17-OHCS		17 KS		17-KGS	
	Control day	Nor adro-stenolon day	Control day	Nor adro-stenolon day	Control day	Nor dro-stenolon day	Control day	Nor adro-stenolon day	Control day	Nor adro-stenolon day
Control patients	17 4 ± 1.00 (30)	15.8 ± 1 01 (31)	33.1 ± 2.66 (14)	31 7 ± 2.96 (13)	7 6 ± 0.97 (26)	8.3 ± 1 49 (18)	3.6 ± 0.38 (28)	4 7 ± 0.78 (20)	14.7 ± 1 45 (28)	16 7 ± 2.56 (23)
Uremic patients	17 0 ± 1 43 (25)	17.5 ± 1 49 (23)	56.0 ± 6.48 (17)	61.3 ± 9.6 (17)	7 7 ± 2.42 (15)	6.8 ± 2.30 (15)	2.2 ± 0.36 (16)	2.6 ± 0.55 (17)	14.5 ± 2.08 (18)	12.6 ± 2.64 (14)

$P < 0.01$ $P < 0.05$

(Figures in brackets indicate number of patients)

ciency than in the controls, 175 5 mg (15) Also after cortisone treatment the plasma 17-OHCS content increased and the urinary excretion of 17 OHCS became less in uremic patients than in the controls (18)

This steroid retention in renal diseases is obviously caused by decreased excretion and not by increased production There is some degree of uncertainty as to the significance of this retention but elevated 17 OHCS in the plasma may lead to the formation of edema and hypertension Because of steroid retention in renal patients it seems advisable to avoid the stimulation of adrenal cortex and the administration of steroid hormones. On the other hand very little is known about the effect of anabolic hormones on the steroid metabolism which led us to study the effect of nor testosterone phenyl propionate (Durabolin ® Organon) on the plasma and urinary steroids in uremic patients.

Material and method

Control blood samples were drawn from 55 patients at 09 00, 10 00 or 12.00 hours. On the following day the same patients were given 50 mg of nor testosterone phenyl propionate (Durabolin ® "Organon) intramuscularly at 08 00 hours and a blood sample was taken at *the same hour as* the control sample of the preceding day Samples were thus obtained 1 2 and 4 hours after the administration of Durabolin Twenty four hour urine was collected on the control day and on the day of Durabolin administration. Thirty-one of the patients with *no symptoms of* renal diseases constituted the control group. Twenty-five patients had renal disease of different severity some of them extremely severe uremia.

The free and conjugated 17-OHCS in the plasma were determined by the method of Peterson and Wyngaarden (19 20) and Thomasson (24) The total 17 OHCS in the urine were determined by the method of Jenkins et al. (7) and Halme et al. (6) 17 KS by a modification of the method of Callow and Zimmerman (Halme et al. 6) The accuracy of the methods has been discussed earlier (16) Urinary 17-ketogenic steroids (17 KGS) were determined by the method of Norymberski et al. (14)

Results

No differences between the control and Durabolin day were established in the urinary excretion or in the plasma level of steroids in the controls or in the uremic patients (table I). The conjugated 17-OHCS in the plasma were twice as high in uremic patients compared with the controls. This retention is known of old and constitutes the point of departure for the present study (8). Nor were any differences demonstrable in the samples taken at different times.

Anabolic steroid, Durabolin, consequently does not stimulate the adrenal cortex or cause steroid retention in the controls, nor does it increase the retention of conjugated 17-OHCS established in uremic patients.

Discussion

The present investigation shows that anabolic steroids have no effect on the 17-OHCS level in the plasma or the urinary excretion of 17-OHCS, 17 KS and 17 KGS. The breakdown of anabolic hormones in the organism occurs along other routes without causing changes in the amounts of steroids examined. Nor did the dose used cause any activation of the adrenal cortex. This is suggested also by the investigations of Rinne and Naatanen (21) on rats in which a small dose of anabolic steroid caused adrenal atrophy, a medium-sized dose produced no effect and large doses caused hypertrophy of the adrenal gland. The present authors were unable to confirm the observation by Brochner-Mortensen (2) that anabolic steroid reduces the 17-OHCS level of the plasma and the 17 KS content of the urine.

As no steroid retention occurs, Durabolin is probably suitable for both acute and chronic renal failure. The subjective well-being of the patients is improved and a parallel drop occurs in the retention of N. P. N. Testosterone, on the other hand, causes retention of 17 KS (15) and therefore the use of anabolic hormones in renal failure is preferable to that of testosterone.

Summary

No changes were established in 31 control patients and 25 uremic patients in the urinary excretion of 17-hydroxycortico-, 17 keto-, 17-ketogenic steroids or in the plasma 17-hydroxycorticosteroid level following the administration of anabolic steroid nor testosterone phenyl propionate (Durabolin) 50 mg per day. It neither stimulated the adrenal cortex nor increased the steroid retention of the uremic patients. It was confirmed in the patients with renal insufficiency that there was a marked retention of conjugated steroids in the plasma. The mean plasma content of conjugated 17-OHCS in the control group was 33 ± 2.66 μg/100 cm³ and in uremic patients 56 ± 6.51 μg/100 cm³.

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Auto-Immune Haemolytic Anaemia in Systemic Lupus Erythematosus

By

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That auto-immune haemolytic anaemia may be the predominant, and in some cases even th first sign of systemic lupus erythematosus (S. L. E.) was not realized until about 10 years ago. The explanation is presumably that until recently S. L. E. was seldom diagnosed. Although the disease has been known for almost a hundred years, the knowledge of its features has been considerably extended after the demonstration of the L. E. cell (1948) Hundreds of cases have been observed since then. Lastly Coombs direct test, introduced into clinical practice in 1945 has been a factor of significance in diagnosing uto-immune haemolytic anemia and has increased the attention given to the haemolytic syndrome

Although anaemia is one of the most common findings in S. L. E. (16 17) auto-immune haemolytic anaemia was considered an unusual complication. During the years after 1950 therefore, reports of single cases were published, in 1951 (9 17 18) 1952 (7) 1953 (1 13 14) 1955 (10, 11 23) and 1956 (2 6)

These reports afford numerous examples that severe auto-immune haemolytic anaemia may be an early and perhaps the only sign of S. L. E. It has been emphasized also that for this very reason reserve should be displayed in making a diagnosis of idiopathic" uto-immune haemolytic anaemia (2) The haemolytic anaemia is in rare cases accompanied by severe thrombocytopenia (9) or else thrombocytopenia may precede auto-immune haemolytic anaemia (4) just as auto-immune haemolysis may be the first sign of S. L. E.

The incidence of auto-immune haemolytic anaemia n S. L. E. ppears to be low However it was observed by Dubois (7) in 6 out of 9 cases, whereas Piscotta et al. (18) found i in only one out of 7 Michael et al. s series comprises 111 patients with clinical S. L. E. but only 3 had haemolytic anaemia (17) Lee & Davis (15) have reported an incidence of uto-immune haemolytic anaemia of about 5 %, usually as an early manifestation. Out of series of 34 patients with

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S. L. E. 11 had haemoglobin levels below 9 g/100 ml, but only 3 of these had auto-immune haemolysis.

As a rule the Coombs direct test is positive in cases of S. L. E. with auto-immune haemolysis (int. al. 9, 15, 18) but a positive Coombs test is not interpreted as an infallible sign that haemolytic anaemia is present (1, 9). According to the available data, however, not sufficient investigation has been undertaken to decide whether a haemolytic syndrome, albeit well compensated, has not been present in these cases. In Dacie's series of 9 patients with S. L. E. Coombs test was strongly positive in only one with pronounced haemolysis and only faintly positive in 5 out of 8 patients "without obvious haemolytic anaemia". As a rule, the antibody is of the "warm" type (15) but some patients also or exclusively show "cold" antibody (12, 15, 18) which may be of high titre (17). Wasserman et al. (23) report that in cases of S. L. E. the Coombs test is frequently positive. This applied to 31 out of their 72 patients but only 6 had "demonstrable haemolytic anaemia". One patient with haemolytic anaemia had a negative Coombs test, but a cold agglutinin with a titre of 320. In 3 of the cases haemolysis was the first sign, but in the others the haemolytic anaemia started 1 1/2, 2, 3 and 3 years after S. L. E. had been diagnosed. Two out of the 6 patients with a positive Coombs test also had a cold agglutinin with titres of 64 and 320.

The treatment of the haemolytic complication appears to have undergone the same development as that of primary auto-immune haemolytic anaemia. In the course of time corticosteroids have acquired decisive importance, while splenectomy has been forced rather into the background. Not all patients give satisfactory response to treatment with corticosteroids, however, then splenectomy has been performed. Perhaps the steroid has been given in too low doses and not for a sufficient length of time. Warnings have been given against splenectomy because in 3 cases exacerbation of the S. L. E. has been observed after the operation (6). One of these patients, however, also had thrombocytopenia and therefore perhaps *a priori* a poor prognosis. In the other two the condition did not deteriorate until months after the operation. Indeed, many patients have been splenectomized without any exacerbation of the underlying disease (7, 9), and in a few cases the haemolytic condition has improved (2, 7, 19).

Material

Since auto-immune haemolytic anaemia is believed to be a fairly uncommon complication in S. L. E., brief reports will be given of 10 patients, several of whom have been under supervision for years.

Case 1 (H. K., P. 15662). A girl, aged 12, was suddenly found to have a Hb. of 7 g/100 ml, while 48 % of the cells in the sternal marrow were normoblasts. E. S. R. 120 mm, spleen distinctly enlarged. The haemoglobin level rose spontaneously to 12 g/100 ml. Subsequently similar episodes of severe anaemia which subsided spontaneously within two weeks. In July 1959 the Hb. was 5.8 g/100 ml. As repeated transfusions were ineffective the spleen was removed, but without any effect upon the Hb. level, reticulocyte count, and the result of Coombs' test. The patient now showed all signs of Addison's disease. On cortisone, Percorten, and prednisone medication the Hb. rose to normal values, and Coombs' test became negative within 6 months. The E. S. R. dropped from 144 mm to normal. The cold agglutinin titre was not elevated. There were L. E. cells and elevated γ-globulin. Alive and working at the end of 4 years.

Case 2 (J. M. 14047) A girl, aged 15, who suddenly developed severe anaemia (Hb. 3.5 g/100 ml) serum bilirubin 4.2 mg/100 ml, reticulocytes 10—13 %, E. S. R. 155 mm, leukocytes 5,600, platelets 180,000, W. R. positive but Nelson test negative. Cold agglutinin titre 128. Coombs' direct test highly positive and remained so for almost 2 years. Not until then did she exhibit typical facial rash and few weeks later swelling and pain in several joints (cf. fig. 2) Throughout the observation period of 3 years she has had latent or manifest haemolytic anaemia. In September 1959 the patient stopped taking prednisone for a few days, and a haemolytic crisis occurred. Increasing the dose of prednisone for short time from 10 to 80 mg daily was enough to reduce the Hb. values rapidly to normal (fig. 2) I January 1960 the patient developed panaritium accompanied by manifest jaundice and an increase in the reticulocyte count. Alive and working at the end of 3 years.

Case 3 (V. R. A. 15265) A girl, aged 17 with migratory arthropathy. Four and half years later facial rash, haemolytic anaemia, markedly increased E. S. R., Coombs' direct test faintly positive. In addition, nephrotic syndrome. Leukocytes 3,300, platelets 7,800. The condition rapidly deteriorated despite 200—400 mg prednisone daily. This rendered the Coombs test negative, but the haemolysis remained pronounced, and the patient died, severely oedematous, at the age of 22. Autopsy confirmed the diagnosis of S. L. E.

Case 4 (A. K. 1649) A girl, aged 17 who had been found to have positive Wassermann and elevated E. S. R. in 1946. Treated as case of lues ignota with arsphenamine and bismuth. Jaundice, which occurred shortly after was interpreted as consequence of the arsphenamine therapy. However the sedimentation rate remained high for the subsequent years, and 4 years later she was found to have severe haemolysis with jaundice, splenomegaly and false positive W. R. The Coombs direct test was positive through 7 1/2 years. Splenectomized in 1951 (weight of spleen 360 g). For the next three years the haemoglobin level was approx. 12—13 g/100 ml, but the E. S. R. remained high and Coombs test positive. Not until 6 years later did she develop arthropathy later pericarditis, pleurisy, nephropathy and terminally cerebral thrombosis. During the last months, when the patient was treated with prednisone, the Coombs' direct test became negative. *Died in 1955, 10 years after the onset of the first sign.* Autopsy confirmed the diagnosis of S. L. E.

Case (A. J. 9873) A 17-year-old girl developed "rheumatoid arthritis" At 19 she began to have facial rash which persisted for 2 years. Not until the age of 21 did she show severe haemolytic anaemia with a faintly positive Coombs direct test, E. S. R. 137 mm, leukocytes 2,200, platelets 8,000. On prednisone, 40 mg daily the haemolysis was controlled for 4 months, but then severe nephropathy and endocarditis set in, and death occurred little more than 5 years after the first sign. Autopsy confirmed the diagnosis of S. L. E. or thrombotic thrombopenic purpura.

Case 6 (T. L. 14705) The disease began as jaundice, E. S. R. 57 mm, γ-globulin 1.68 g/100 ml, + L. E. cells, and mild haemolytic anaemia in a 19-year-old girl. Coombs direct test faintly positive cold agglutinin titre 64 increased osmotic fragility leukocytes 7 400, platelets 160 000 Slight arthropathy fatigue and transitory oedema of the face. Some improvement on chloroquine diphosphate Coombs test became negative, the E. S. R. dropped, but the jaundice persisted. There was no anaemia worth mentioning Alive and working almost 4 years after the first sign.

Case 7 (R. K. O. 9947) Rather suddenly 27 year-old woman developed severe auto-*immune* haemolytic anaemia, Hb. 7.9 g/100 ml, serum bilirubin 2.5 mg/100 ml, E. S. R. 95 mm, leukocytes 2,000, platelets 152,000. Coombs' direct test moderately positive no cold agglutinins, but pronounced Raynaud syndrome Coombs direct test remained positive for at least 18 months, but during the subsequent 3 years it was negative After the disease had persisted for 3 years, she developed arthropathy and the subsequent year malignant hypertension and fatal cardiac failure. Autopsy revealed the renal changes characteristic of S. L. E. and pericarditis.

Case 8 (K. M. K. 10919) A 28-year-old woman developed "rheumatoid arthritis Two years later muscular pain, episodes of fever and facial rash. One month later the Hb was 8.0 g/100 ml, leukocytes 1,600, platelets 7 700, E. S. R. 137 mm, Coombs direct test moderately positive through period of 3

months while the E. S. R. was particularly high. On prednisone, 20—30 mg daily the Coombs test returned to negative in 6 weeks and the Hb rose from 8 to 11—12 g/100 ml. Since then no haemolytic episodes. She is alive and working 5 years after the first sign and has been feeling well during the past 2 years.

Case 9 (N M P 17165) In a 49-year-old woman the first sign was classical rheumatoid arthritis. Fifteen years later repeated episodes of fever and attacks of auto-immune haemolytic anaemia with elevated cold agglutinin titre but a negative Coombs direct test. W R. false positive, + L. E. cells, leukocytes 8,000, platelets 154 000 During prednisone medication a marked increase in Hb. decrease of E. S. R., whil the cold agglutinin titre remained unaffected. Alive and working 18 months after the onset of haemolytic anaemia.

Case 10 (A. A. M C. 9849) A 51 year-old woman developed muscular and articular pain. Six and a half years later the Coombs direct test was moderately positive, the E. S R. rising Hb. falling while the reticulocyte count was seldom elevated. On prednisone therapy the E. S R. dropped, and after the Coombs direct test had returned to negative in one year the Hb went up. In the spring of 1960 the medication was interrupted and the E. S. R. increased considerably the Hb. dropped, and Coombs direct test again became positive. Alive with muscular and articular pain.

Results

Among 24 consecutive definite cases of S L. E. diagnosed during the period 1951 —1960 on the basis of obvious clinical appearances laboratory tests showing typical L. E cells or autopsy showing typical microscopic changes in the organs, auto-immune haemolytic anaemia was found during the course of the disease in 9 or about 40 % of those with classical S. L. E. The explanation of this relatively high incidence is presumably in part that the department has admitted a relatively large number of patients with anaemia

The series includes also a case of collagenosis, very probably S L. E. (case 10) The criteria of auto-immune haemolytic anaemia have been anaemia and signs of hyperhaemolysis combined with a positive direct Coombs test and/or elevated cold agglutinin titre.

Haemolysis in relation to the course of the basic disease

The brief case histories of the 10 patients all of whom are women and the majority under 30 years of age, illustrate the diversity of the symptoms and signs. They show also that haemolytic anaemia may be a dominant or a more subordinate sign in S. L. E. Fig 1 illustrates how auto-immune haemolysis may be transitory or persist for several years. It shows also at what stage of the disease haemolysis was observed. In 5 instances auto-immune haemolytic anaemia was the first manifestation of S L. E. In several of these cases months or years elapsed before other signs of S L. E. appeared Fig 2 exemplifies how auto-immune haemolytic anaemia had to be classified — for more than two years — as primary or idiopathic (case 2) It was only after the lapse of this period that the patient exhibited a typical rash on the cheeks and nose. At approximately the same time the developed migratory arthropathy and the diagnosis of S L. E could be made. Throughout the observation period of about 3 years, however the predominant sign was latent or manifest haemolytic anaemia, with a positive Coombs direct test In case 4 it was 6 years and in case 7 three years before it was realized that the basic disease was S. L. E.

It will be seen that in the present series auto-immune haemolytic anaemia was far

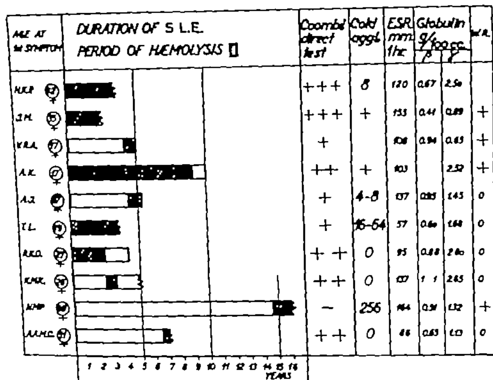


Fig. 1 Survey on periods of haemolysis in the course of the 10 cases of systemic lupus erythematosus. Data regarding serum proteins

from uncommon, sometimes a prolonged and predominant clinical feature in S. L. E. Apparently it is due to chance in which cases, when, and for how long the immunizing processes are directed t the patient s erythrocytes and thus give rise to haemolytic anaemia. The anaemia may be severe, and it may be chronic and even manifest tself as haemolytic crises (fig. 2) Moderate splenomegaly was the rule The E. S. R. was invariably very much elevated during the haemolytic phases. Frequently there was a moderate or marked elevation of β- and γ-globulins (cf. fig. 1) and the W R. was obviously a "false positive in 4 out of 9 cases. As a further sign of immunization, the patients occasionally howed leukocyte and platelet agglutinins. On the other hand, abnormal iso-antibodies were not observed in this series.

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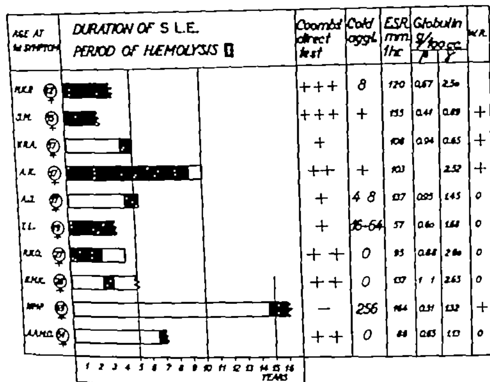


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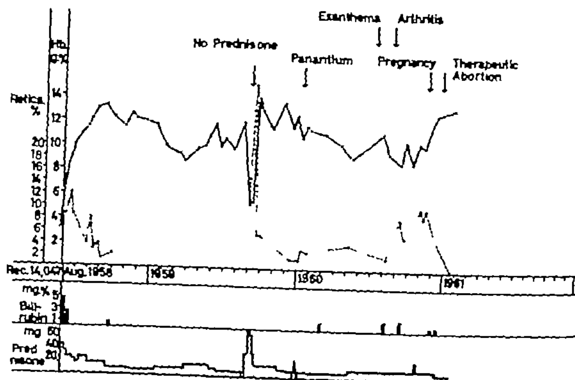


Fig 2 Variations in Hb., reticulocytes, degree of jaundice, and sequence of signs in a young woman (case 7) with severe auto-immune haemolytic anaemia. For 2 years the condition had been interpreted as primary auto-immune haemolysis until it was realized that it was a link in S. L. E.

Haemolysis in relation to Coombs' direct test

As shown in fig 1 Coombs test was positive in 9 cases. In case 9 it was negative, while the cold agglutinin titre was increased to 256 In another 2 patients (cases 2 and 6) not only was the Coombs direct test positive, but the cold agglutinin titre was slightly elevated as well (to 128 and 64 respectively) In two instances (cases 4 and 7) the Coombs direct test returned to negative again, in case 4 after long term prednisone medication, but in case 7 without any particular treatment.

Whenever the Coombs test was positive there were massive or mild signs of hyperhaemolysis, and the sedimentation rate was invariably usually greatly increased During periods with a negative Coombs test, the sedimentation rate was nevertheless often highly increased — incidentally one of the most common findings in active S L. E. whether or not it is accompanied by haemolytic anaemia (16) When the treatment entailed decisive reduction in haemolytic activity the sedimentation rate often fell considerably it is true but unlike the general findings in the treatment of primary auto-immune haemolytic anaemia it was seldom normal on corticosteroids.

There was no difference in clinical appearances between patients with elevated cold agglutinin titre — demonstrated only in a fairly low titre — and patients without such elevation Without having demonstrable cold agglutinins case 7 exhibited a typical Raynaud syndrome, while the three patients with a slightly elevated cold agglutinin titre did not have a Raynaud syndrome.

Treatment

Prednisone has been an important therapeutic agent, while splenectomy — carried out in only 2 cases — appears to have been of less importance. Case 1 had splenectomy without the slightest effect upon the haemolytic activity E. S. R., or the result of Coombs test. On the other hand, corticosteroid medication instituted at a later date entailed an increase in Hb. of from 6 to 14 g/100 ml and reduced the E. S. R. to normal. Case 4 also had a splenectomy For several months after the operation the Hb. was 2 g/100 ml higher than before, while the Coombs test and E. S. R. remained unchanged. Three or four years later this patient had a haemolytic crisis which was easily controlled with ACTH. After permanent prednisone medication was instituted, because of nephropathy and carditis, the Hb. has been high and the Coombs direct test has become negative.

Thus, splenectomy has had only a doubtful effect upon the haemolytic condition and does not seem to have influenced the course of the basic disease t all.

On the other hand, treatment with prednisone has had convincing effect in several of the cases. In case 2 prednisone medication, 20—40 mg daily has been required for 3 years (fig 2) a decrease in the dosage or interruption of the medication has immediately resulted in anaemia, just as in some patients with primary auto-immune haemolytic anaemia (22) In cases 5, 8, and 9 who had severe haemolytic anaemia, the degree of haemolysis was immediately diminished after institution of prednisone. In case 3 prednisone in doses of up to 400 mg daily for 2 months failed to affect the degree of haemolysis, but this patient had thrombocytopenia as well as severe nephropathy In three cases of auto-immune haemolytic anaemia (cases 4 6, and 7) the haemolysis was well compensated and for long periods accompanied by jaundice, but there was only one crisis. Treatment of the latter was not instituted, and it also proved to be unnecessary Case 4 was splenectomized, but without convincing effect. In these cases the auto-immune haemolysis must be designated as chronic, not needing treatment. Case 10 was treated for 2 years with prednisone without convincing effect upon the anaemia. However Coombs test turned negative, and when finally prednisone was withdrawn Coombs test again became positive and the Hb fell. In acute cases with severe anaemia prednisone is thus indicated, although one cannot be sure that even large doses will always be effective. Some cases having well-compensated chronic haemolysis do not appear to require treatment.

Summary and conclusion

Judging by the literature, auto-immune haemolytic anaemia is by now a well-known, but rare phenomenon in systemic lupus erythematosus (S. L. E.)

Ten cases of S L. E. complicated by symptomatic, auto-immune haemolytic anaemia are briefly described. In 40 out of 24 consecutive patients with classical S L. E. the haemolysis was of several months and sometimes of several years duration. In 5 cases sever haemolysis was the initial sign, and it was not until 6 years later that t was realized that the haemolysis was a sign of S. L. E. Until then, the disease had been interpreted as primary auto-immune haemolytic anaemia. In the other 5 cases the haemolysis appeared years, in some of

them many years after other signs of S. L. E. had set in.

This secondary haemolytic anaemia was often chronic, of several years duration but in one instance it was merely a transitory episode lasting for a few months.

Nine patients had a positive direct Coombs test and 2 moreover an elevated cold agglutinin titre. One patient had a negative Coombs test but a constantly elevated cold agglutinin titre. When the Coombs direct test was positive, there were always more or less marked signs of hyperhaemolysis, but Coombs direct test was not always positive despite evidence of haemolytic anaemia.

Prednisone had a striking effect in 3 cases of severe haemolytic anaemia of fairly acute onset. In a fourth case of the same category however even high doses were ineffective.

In 3 cases the symptomatic auto-immune haemolytic anaemia was chronic and well-compensated therefore, it was not treated. In one case, which so far has lasted for 3 years, prednisone therapy could not be discontinued.

Two patients were splenectomized without convincing effect.

Acknowledgement

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From the Medical Clinic of the Central Hospital (Head Pekka Brummer M. D.) and from the Department of Medical Microbiology (Head Eero Mustakallio, M. D.)
University of Turku, Finland

Riboflavin Content of Blood in Carriers of Fish Tapeworm (Diphyllobothrium latum)

By

Toivo Markkanen

The theory of v. Bonsdorff (1, 2, 3) is well-known, i. e. that the host and the parasite (diphyllobothrium latum) compete for vitamin B_{12}. A deficiency of B_{12} may cause megaloblastic anemia in the host, especially if the tapeworm is situated high up in the small intestine. Thus most reports published concern the correlation between vitamin B_{12} and fish tapeworm, or fish tapeworm anemia. However it is not illogical to assume that the fish tapeworm might disturb also the nutritional state of other vitamins in the host. A previous report from our clinic mentioned that in carriers of fish tapeworm, the urinary excretion of thiamine, riboflavin, pantothenic acid and biotin is decreased as compared with healthy controls (4). In the following the aim is to study the vitamin B-level of blood in patients with broad fish tapeworm. At first, in the present investigation the riboflavin content of blood of these patients has been analysed.

Submitted for publication August 18, 1961

Material and method

The material included 80 patients. 20 of these were carriers of broad fish tapeworm (table II) and the other 60 patients served as a control group (table I). Of the fish tapeworm carriers 9 had megaloblastic anemia (verified by sternal puncture and by blood examinations) and two hypochromic anemia (Nos 13 and 18 in table II). In one case (No. 14 in table II) the riboflavin content, Hb, and blood cell values were studied before and after tapeworm eviction (fig. 1). Specimens for venous blood analyses were taken in the morning before intake of food. In all cases the presence of fish tapeworm was verified by the finding of worm eggs in the feces. All the blood samples for riboflavin determination were analysed microbiologically (5) using *L. casei* ATCC 7469 as the test organism.

In the statistical analysis of the results t, standard deviation and standard error of the mean (SEM) were calculated. When $p < 0.001$ it is said that the difference is highly significant.

Aided by a grant from the Sigrid Jusélius Foundation.

them many years after other signs of S L. E had set in.

Thus secondary haemolytic anaemia was often chronic, of several years duration but in one instance it was merely a transitory episode lasting for a few months.

Nine patients had a positive direct Coombs test and 2 moreover an elevated cold agglutinin titre. One patient had a negative Coombs test but a constantly elevated cold agglutinin titre. When the Coombs direct test was positive, there were always more or less marked signs of hyperhaemolysis, but Coombs direct test was not always positive despite evidence of haemolytic anaemia

Prednisone had a striking effect in 3 cases of severe haemolytic anaemia of fairly acute onset. In a fourth case of the same category however even high doses were ineffective.

In 3 cases the symptomatic auto-immune haemolytic anaemia was chronic and well-compensated therefore it was not treated In one case which so far has lasted for 3 years, prednisone therapy could not be discontinued

Two patients were splenectomized without convincing effect

Acknowledgement

I acknowledge my indebtedness to Dr E. Freiesleben, Head of the Hospital Blood Bank, for valuable discussions and for having performed most of the Coombs tests.

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Results

The results for controls and test patients are given in tables I and II. As seen, the average for controls is 9.3 ± 0.25 μg per cent and for fish tapeworm carriers 6.4 ± 0.33 μg per cent. The difference is statistically highly significant ($p < 0.001$). There are low riboflavin values both in anemic and non-anemic patients.

In fig. I the blood riboflavin, hemoglobin, red and white cell count in test patient No 14 (table II) on some observation days over a period of about two months are reported. This patient was hospitalized, and the tapeworm was evicted three days after the control blood analyses. During the following three weeks blood samples for Hb, red and white cell count and for riboflavin analyses were taken in the hospital. The patient was then sent home for a month and returned to hospital for final observation. During these two months of observation, the riboflavin content in the blood ranged from 4.0 μg per cent to 12.3 μg per cent. At the same time the Hb increased 2.7 times, the W. B. C.-count 2.9 and the R. B. C. count 3.2 times, as calculated from their original values before tapeworm eviction.

Table II. Fish tapeworm carriers. Blood cell values and corresponding riboflavin content in whole blood (μg %)

No.	Sex	Age	Hb g %	R.B.C. mill. per mm³	W.B.C. thous. per mm³	Riboflavin μg %
1	M	62	15.7	5.18	4.8	7.6
2	M	23	16.6	5.30	5.2	5.8
3	F	58	14.3	4.44	7.7	5.4
4	F	62	12.8	4.50	4.8	5.3
5	F	57	13.4	4.30	7.2	5.3
6	F	41	13.9	4.20	4.2	6.2
7	F	47	12.6	3.98	5.9	6.3
8	F	74	13.4	4.51	6.5	7.0
9	F	40	9.9	2.77	4.4	6.1
10	M	69	9.6	2.57	8.4	9.4
11	M	59	6.1	1.33	4.1	8.2
12	F	65	12.5	4.48	6.1	7.0
13	F	18	5.6	3.17	5.5	6.2
14	M	30	5.5	1.30	2.0	4.0
15	F	53	7.1	1.73	1.3	6.2
16	M	65	5.5	1.52	3.4	8.3
17	M	52	8.7	2.06	4.9	6.3
18	F	20	9.4	4.50	6.9	9.4
19	M	73	7.8	1.57	4.6	4.7
20	F	62	7.6	1.35	3.8	8.1
					Mean	6.4
					Stand. dev	1.5
					SEM	± 0.33

Treated with liver extract before examination.
Hypochromic anaemia.

Discussion

In previous investigation (4) concerning urinary excretion of vitamins B a significant decrease in the excretion of thiamine and pantothenic acid has been observed. In anemic tapeworm carriers decreased excretion was likewise noted with regard to riboflavin, although the decrease was not statistically significant. The riboflavin analyses now performed in whole blood, showed a distinct decrease of the vitamin mentioned in anemic as well as in non-anemic patients with tapeworm.

It is known that the blood cells contain the greatest part of total riboflavin as so-called flavin-adenin-dinucleotids (FAD). This amount is greater in the white cells than in the red cells. In this investigation, however, there were low riboflavin values both in test patients with normal blood cell count and in those which had low W. B. C. and R. B. C. values. Fractional analysis by which the riboflavin content in plasma or in serum, and in red and white cells might be distinguished, would yield more reliable result of the true status of riboflavin in the blood.

Table I Control patients for blood riboflavin examination. Riboflavin content in µg per cent

No.	Sex	Age	Diagnosis	Riboflavin µg %
1	M	54	Stenocardia	7.0
2	M	42	Fibrillatio atriorum cordis	9.0
3	M	59	Insultus cerebri	10.0
4	M	41	Status post laparatomiam explor	10.0
5	M	54	Stenocardia	12.5
6	M	59	Apoplexia cerebri	8.6
7	M	59	Venectasiae haemorrh.	8.6
8	M	28	Arthritis gen. sin.	8.1
9	M	68	Aneurysma aortae luetica	9.4
10	M	59	Nihil obj.	7.8
11	M	66	Vertigo	8.5
12	M	68	Emphysema pulmonum	12.4
13	M	55	Emphysema pulmonum. Stenocardia	10.2
14	M	17	Albuminuria	8.1
15	M	47	S enocardia	12.3
16	M	54	Status post infarct. cordis	13.7
17	M	51	Insuf. cordis	11.6
18	M	40	Stenocardia	12.6
19	M	55	Asthma bronchiale	7.4
20	M	52	Infarctus cordis. Sinuitis max.	8.5
21	M	45	Stenocardia	7.3
22	M	50	Carcinoma pulm. dx.	7.9
23	M	64	Emphysema pulmonum. Tub. pulm. sanata	9.7
24	M	65	Status po t pneumoniam	11.2
25	M	60	Arterio clerosis vasorum medul. spin.	9.1
26	M	60	Arteriosclerosis vasor cerebri	8.5
27	M	60	Trombosis art. cerebri. Emphysema pulm.	7.6
28	M	40	Epilepsia. Ulcus cruris	9.8
29	M	71	Status post nephrectomiam	12.5
30	M	66	Stenocardia. *Insuf.* cordis	10.9
31	M	61	Arteriosclerosis universalis	13.6
32	M	49	Status post infarct. cordis	1 .5
33	M	59	Stenocardia	9.6
34	M	51	Status post infarc cordis	8.5
35	M	53	Nihil obj.	7.6
36	M	47	Status post infarct. cordis	6.4
37	M	55	Status post infarct. cordis	9.8
38	M	52	Haemorrhagia subarachnoidalis	7.5
39	M	61	Status post infarct. cordis	10.6
40	M	45	Hypertensio art. maligna	10.8
41	M	37	Status post infarct. cordis	9.7
42	M	52	Emphysema pulmonum. Claudicatio	9.0
43	M	67	Stenocardia	11.3
44	M	42	Hypertensio art.	8.1
45	F	80	Struma non toxica	8.8
46	F	72	Stenocardia	7.4
47	M	51	Intoxicatio barbit.	9.1
48	M	50	Nihil obj.	9.3
49	M	50	Arthritis rheum. incipiens?	9.0
50	M	51	Anaemia hemolytica curata	9.6
51	M	49	Stenocardia. Cholelithiasis	9.9
52	M	44	Stenocardia. Arthritis rheum.	8.0
53	F	69	Encephalopathia arteriosclerotica	6.8
54	F	72	Stenocardia	7.4
55	F	62	Emphysema pulm. Cor pulmonale	8.4
56	M	54	Abscessus pulm.	9.3
57	M	67	Status post infarct. cordis	7.5
58	F	62	Cor pulmonale	8.4
59	F	51	Hyperthyreosis?	7.9
60	F	55	Arthritis rheum.	10.2
			Mean	9.3
			Stand. deviation	1.9
			SEM	± 0.25

Summary

The riboflavin content in whole blood was microbiologically studied in 60 controls and 20 anemic or non-anemic carriers of fish tapeworm. The average riboflavin content in the controls was 9.3 ± 0.25 μg per cent and in the tapeworm carriers 6.4 ± 0.33 μg per cent. The difference is statistically highly significant.

Acknowledgements

My thanks are due to Mr. Esa Abo, Ph. D for giving me permission to obtain assistance from his technical staff at the Pharmaceutical Manufacturers Leiras and for placing the laboratory material required for this investigation at my disposal.

I am also indebted to Dr Petter Savola, M. D., for procuring some of the blood samples for my study

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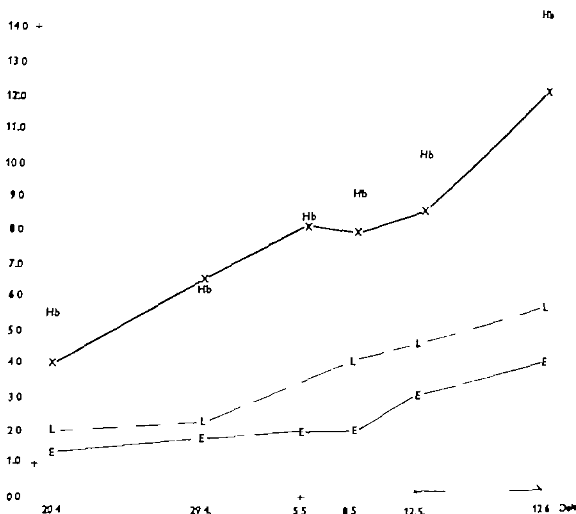


Fig 1 Curves for blood riboflavin, Hb, erythrocytes and leucocytes in patient No. 14 (cf table II) before and after fish tapeworm eviction (↓)

X = Blood riboflavin μg% E = Erythrocytes mill. per mm³ L = Leucocytes thous. per mm
Hb = Hemoglobin g%

Clinical experience shows that regeneration of blood does not start until about 1—2 weeks after eviction of the tapeworm. It is possible that during this period filling of the exhausted depots in the organism thus occurs and not until in the certain phase of this process does, for instance, regeneration of blood set in Also the comparatively large increase in the riboflavin content in blood in the two weeks after eviction of the tapeworm substantiates the clinical observation When the full regeneration of the patient's blood had taken place after two months, the increase in riboflavin was 3 1 times greater in red cells 3 2, in white cells 2 9 and in hemoglobin 2 7 times, as compared with the initial values before tapeworm eviction (fig 1) During this time, reached also the urinary excretion of vitamin B_{12} and folic acid the normal average.

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Aus der Medizinischen Klinik der Universität Bern (Direktor: W. Hadorn, Dr. med.)
Bern, Schweiz

Akute Nebenreaktionen auf Penicillinpräparate[1]

von

R. Hoigné

Die akuten Nebenerscheinungen auf Medikamente treten meist kurze Zeit nach der Verabreichung des Arzneimittels auf. Sie werden deshalb ohne weiteres als solche erkannt. Je akuter das Geschehen, desto grösser ist die Schwierigkeit, die einzelnen Ereignisse zu erfassen. Darin dürfte einer der Gründe liegen, weshalb die Untersuchung der akuten Nebenwirkungen von Arzneimitteln lange vernachlässigt wurde. Die Frage fand erst in den letzten Jahren höchste Beachtung, als sich die Erkenntnis verbreitete, dass Reaktionen gegen Arzneimittel vorkommen, welche in jeder Hinsicht dem anaphylaktischen Schock gegen artfremde Eiweisse entsprechen. Beeindruckt durch bedrohliche anaphylaktische Reaktionen an eigenen Patienten, drängte sich eine sorgfältige Differenzierung der verschiedenen akuten Nebenerscheinungen gegen Medikamente in imperativer Weise auf.

Die akuten Nebenwirkungen von Penicillin und Penicillindepot-Präparaten stellen lediglich ein Beispiel solcher Situationen dar. Dabei konnten zwei Gruppen von Reaktionen unterschieden werden: Reaktionen vom allergischen Typ — im Vordergrund die Kreislaufsymptome, Ödeme, ev. Exantheme — und Reaktionen nichtallergischer Art, beherrscht durch akustische und optische Erscheinungen, die bisher ausschliesslich im Zusammenhang mit der Verabreichung von Depot-Präparaten festgestellt wurden. Durch sorgfältige klinische Beobachtung der Reaktion und durch anamnestische Erhebungen gelingt für die Mehrzahl der akuten Allgemeinreaktionen die Zuordnung in eine der beiden pathogenetisch verschiedenen Gruppen.

Anaphylaktischer Schock

Im Bestreben, eine möglichst klare Differentialdiagnose vornehmen zu können, werden zunächst die Symptome beim anaphylaktischen Schock auf Penicillin, wie sie bei 17 Patienten festgestellt wurden, zusammengefasst. Es handelt sich dabei um Fälle, die lediglich Penicillin (Penicillin G oder V) erhielten (Tab. I).

Bei der Redaktion am 17. August 1961 eingegangen.

[1] Die Abbildung und die Tabellen dieser Arbeit wurden anlässlich eines Vortrages am Antibiotica-Symposium in Aachen am 18. und 19. Mai 1961 gezeigt.

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Überblicken wir die Mitteilungen von anaphylaktischem Schock auf verschiedene Penicillinpräparate, so überwiegen in absoluten Zahlen die anaphylaktischen Reaktionen nach Procain-Penicillin. Dies dürfte mit der grossen Verbreitung von Procain-Penicillin im Zusammenhang stehen. Die Allergie ist meistens gegen das Penicillin gerichtet, seltener gegen Penicillin *und* Procain. Auf Grund theoretischer Erwägungen könnte sie auch ausschliesslich den Procainanteil des Moleküls betreffen. Vielversprechend erschien zur Verminderung von Sensibilisierungen gegen Penicillin die Entwicklung eines Kombinationspräparates mit einem Antihistaminicum. Ob unter Antihistamin-Penicillinen die Penicillinallergien, insbesondere die entscheidenden lebensbedrohlichen Reaktionen seltener werden, lässt sich auf empirischer Grundlage noch nicht entscheiden. Allergische Nebenwirkungen werden im Zusammenhang mit der Verabreichung der Antihistaminverbindung noch beobachtet. Da unseres Wissens bisher keine anaphylaktischen Reaktionen nach Verabreichung von Antihistamin-Penicillin beschrieben sind, soll eine vielleicht erste und einzige Beobachtung hier mitgeteilt werden.

Der 62 jährige Patient F. K. erhielt am 4. Juli 1961 wegen eines bronchopneumonischen Schubes und Asthmabronchitis bei Bronchiektasen eine intramuskuläre Injektion von Antihistamin-Penicillin (800,000 E. Allercur Penicillin mit 200,000 E. Benzylpenicillin). Bereits 4 Minuten nach der Injektion Auftreten von *Schlechtwerden, Schwellung* und *Juckreiz im Gesicht, Absinken des Blutdrucks auf 85/60 mm Hg* bei Normalwerten um 150/85 mm Hg, Tachykardie von 100 pro Minute, fast gleichzeitig *generalisierte Urticaria, Zunahme der bronchialasthmatischen Atembehinderung* und *Schwindelgefühl*.

Zur Behandlung wurden 2 mg Noradrenalin in laufender Infusion sowie 3 × 25 mg eines injizierbaren Corticoidpräparates, die erst ... Dosis sofort in eine Vene, verabreicht ... ausserdem erhielt der Patient 3 × 2 ml eines Kampferpräparates intramuskulär.

Tabelle II. Nicht allergische Reaktion auf Procain-Penicillin

Symptome	24 eigene Beobachtungen	12 der 24 Fälle mit fortgesetzter Penicillinanwendung
Akustische Erscheinungen	21	9
Ohrensausen, Brummen, Rauschen	10	4
Überlaute, unwirkliche Töne	7	3
Hyperakusis	3	1
Hypakusis	5	3
Schwindelgefühl	12	7
Sehstörungen	11	7
Doppeltsehen	1	0
Dunkel- und Lichtempfindungen	2	2
Flimmern	4	3
Regenbogenfarben	2	2
Unscharfes Sehen	2	0
Verzerrung der Gegenstände	1	1
Geschmacksempfindungen	5	2
Parästhesien	6	4
Verwirrungszustand	5	2
Angst, Todesangst	17	7
Engigkeitsgefühl, Atemnot	6	2
Herzklopfen und oder Tachykardie	9	5
Blutdrucksteigerung	2	2
Blässe	3	2
Cyanose	2	2
Übelkeit	2	2
Schweissausbruch	2	1
Wiederholtes An- und Abschwellen der Symptome	2	0

Im Verlauf von 12 Stunden trat vollständige Erholung ein.

Zur Sensibilisierung kam es wahrscheinlich im Verlaufe der letzten 4 Jahre, während denen der Patient gesamthaft mit 15.2 Mill. E. Penicillin behandelt wurde.

Tabelle 1 Anaphylaktischer Schock auf Penicillin

Zahl der Fälle	17
Kreislauf	
Blutdruckabfall	15
Puls nicht fühlbar	6
tachycard	7
bradycard	1
Gesichtsfarbe	
Cyanose	8
Blässe	2
Bewusstseinsverlust	8
Atmung	
Atemnot	11
(davon *Asthma bronch.* 3)	
Apnoe	1
Oberflächliche Atmung	1
Haut und Schleimhaut	
Ödem von Zunge, Lippen und Laryn	2
Gesichtsödem ohne Exanthem	3
Exanthem (Urticaria, Erythem)	4
Juckreiz ohne Exanthem	2
Schweissausbruch	3
Weitere Symptome	
Erbrechen	2
Nausea	1
Schwindel	1
Eigenartige Geschmacksempfindungen	2
Durchfall	2
Kribbeln auf den Lippen	1

17 Beobachtungen Literatur (Lit.) 4 Fall 1 Lit. 5, Fall 7 Lit. 6 Lit. 8, Fall 1 Lit. 9, Fälle 2, 4 und 6 Lit. 10 Lit. 11 Lit. 12, Fälle 1 und 2 Lit. 13, Fall 3 Lit. 14 Lit. 16, Fall 3 Lit. 17 Lit. 18.

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Unter den Symptomen steht das akute Kreislaufversagen mit Absinken des Blutdrucks an erster Stelle. Trotzdem werden gelegentlich Formen oder Stadien des anaphylaktischen Schocks beobachtet, bei denen der Blutdruckabfall ausbleibt, oder das klinische Schockbild sogar mit einem Blutdruckanstieg einhergeht. Der Puls erfährt meist eine Frequenzänderung häufiger eine Tachykardie als eine Bradykardie. Die Atemnot, deren Pathogenese unter den akuten Bedingungen des anaphylaktischen Schocks kaum gründlich untersucht werden kann, ist nur ausnahmsweise vom bronchialasthmatischen Typ. Sie liesse sich in manchen Fällen durch das Absinken des Blutdrucks erklären. Trotzdem kommt sie auch bei Fehlen von Asthma bronchiale und Kreislaufversagen dann als besonders feines Symptom einer allergischen Allgemeinreaktion, vor Selbstverständlich muss in jedem Fall eine mechanische Behinderung der Atmung durch ein Ödem im Bereich der oberen Luftwege ausgeschlossen werden. Der Eintritt der Bewusstlosigkeit ist wahrscheinlich eine Folge des Versagens von Kreislauf und Atmung

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Überblicken wir die Mitteilungen von anaphylaktischem Schock auf verschiedene Penicillinpräparate, so überwiegen in absoluten Zahlen die anaphylaktischen Reaktionen nach Procain Penicillin. Dies dürfte mit der grossen Verbreitung von Procain-Penicillin im Zusammenhang stehen. Die Allergie ist meistens gegen das Penicillin gerichtet, seltener gegen Penicillin *und* Procain. Auf Grund theoretischer Erwägungen könnte sie auch ausschliesslich den Procainanteil des Moleküls betreffen. Viel versprechend erschien zur Verminderung von Sensibilisierungen gegen Penicillin die Entwicklung eines Kombinationspräparates mit einem Antihistaminicum. Ob unter Antihistamin-Penicillinen die Penicillinallergien, insbesondere die entscheidenden lebensbedrohlichen Reaktionen seltener werden, lässt sich auf empirischer Grundlage noch nicht entscheiden. Allergische Nebenwirkungen werden im Zusammenhang mit der Verabreichung der Antihistaminverbindung noch beobachtet. Da unseres Wissens bisher keine anaphylaktischen Reaktionen nach Verabreichung von Antihistamin-Penicillin beschrieben sind, soll eine vielleicht erste und einzige Beobachtung hier mitgeteilt werden.

Der 62 jährige Patient F. K. erhielt am 4. Juli 1961 wegen eines bronchopneumonischen Schubes und Asthmabronchitis bei Bronchiektasen eine intramuskuläre Injektion von Antihistamin-Penicillin (800,000 E. Allercur Penicillin mit 200,000 E. Benzylpenicillin). Bereits 4 Minuten nach der Injektion Auftreten von *Schleimhautbrennen*, *Schwellung* und *Juckreiz im Gesicht*, *Absinken des Blutdrucks auf 85/60 mm Hg* bei Normalwerten um 150/85 mm Hg, Tachykardie von 100 pro Minute, fast gleichzeitig generalisierte Urticaria, *Zunahme der bronchasthmatischen Atembehinderung* und *Schwindelgefühl*.

Zur Behandlung wurden 2 mg Noradrenalin in intravenöser Infusion sowie 3 × 25 mg eines injizierbaren Cortisonpräparates, die

Tabelle II. Nicht allergische Reaktionen auf Procain-Penicillin

Symptome	24 eigene Beobachtungen	12 der 24 Fälle mit fortgesetzter Penicillinanwendung
Akustische Erscheinungen	21	9
Ohrensausen, Brummen, Rauschen	10	4
Überlaute, unwirkliche Töne	7	3
Hyperakusis	3	1
Hypakusis	5	3
Schwindelgefühl	12	7
Sehstörungen	11	7
Doppeltsehen	1	0
Dunkel- und Lichtempfindungen	2	2
Flimmern	4	3
Regenbogenfarben	2	2
Unscharfes Sehen	2	0
Verzerrung der Gegenstände	1	1
Geschmacksempfindungen	5	2
Parästhesien	6	4
Verwirrungszustand	5	2
Angst, Todesangst	17	7
Engigkeitsgefühl, Atemnot	6	2
Herzklopfen und/oder Tachykardie	9	5
Blutdruckanstieg	2	2
Blässe	3	2
Cyanose	2	2
Übelkeit	2	2
Schweissausbruch	2	1
Wiederholtes An- und Abschwellen der Symptome	2	0

erste Dosis sofort in eine Vene, verabreicht. Ausserdem erhielt der Patient 3 × 2 ml eines Kampferpräparates intramuskulär.

Im Verlaufe von 12 Stunden trat vollständige Erholung ein.

Zur Sensibilisierung kam es wahrscheinlich im Verlauf der letzten 4 Jahre, während denen der Patient gesamthaft mit 15.2 Mill. E. Penicillin behandelt wurde.

Tabelle I Anaphylaktischer Schock auf Penicillin

Zahl der Fälle	17
Kreislauf	
Blutdruckabfall	15
Puls nicht fühlbar	6
tachycard	7
bradycard	1
Gesichtsfarbe	
Cyanose	8
Blässe	2
Bewusstseinsverlust	8
Atmung	
Atemnot	11
(davon Asthma bronch. 3)	
Apnoe	1
Oberflächliche Atmung	1
Haut und Schleimhaut	
Ödem von Zunge, Lippen und Larynx	2
Gesichtsödem ohne Exanthem	5
Exanthem (Urticaria Erythem)	4
Juckreiz ohne Exanthem	2
Schweissausbruch	3
Weitere Symptome	
Erbrechen	2
Nausea	1
Schwindel	1
Eigenartige Geschmacksempfindungen	2
Durchfall	2
„Kribbeln" auf den Lippen	1

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Tabelle III Akute Nebenreaktionen auf Antihistamin-Penicillin (1 Mill. E. i. m.)

Alter Jahre	Zahl der Injektion	Grundkrankheit	Zeit zwischen Injektion und Reaktion	Akute Symptome	Verlauf	Dauer
23	4	Sinusitis	1 Minute	Übelkeit Ohrensausen	wellen-artig	½ Stunde
31	1	unspez. Urethritis	knapp 1 Minute	Ohrgeräusche Übelkeit Blässe		5 Minuten
57	4	Asthma-bronchitis	sofort	Klingen in Ohren, Schwindel, Rötung des Gesichtes, Husten	Abklingen des Schwindels und Hustens in 30'	1½—2 Stunden
48	2	Tonsillitis ac. mit Gelenkerscheinungen	sofort	Ohrenläuten		einige Stunden

Häufigkeit dieser Nebenreaktion kaum vermindern. Mit der alten Technik wurde auf durchschnittlich 720 Mill. E., mit der neuen auf 550 Mill. E. Procain-Penicillin eine der nichtallergischen Reaktionen beobachtet.

In Anbetracht der Gleichzeitigkeit oder kurzfristigen Folge von Injektion und Symptomen liegt die Vermutung nahe, es könnte doch ein Teil des Präparates in eine Vene gelangt sein. Auch Batchelor Horne und Rogerson (1) Björnberg und Selstam (2) sowie Randazzo und Di Prima (15) welche über ähnliche Reaktionen berichten, vertreten diese Auffassung. Ob die zerebralen Reizsymptome durch Procain, durch Verbindungen von Penicillin mit Procain oder auch andern Substanzen zustande kommen, ist noch nicht geklärt. Bei unseren Reaktionen fiel zunächst die grosse Ähnlichkeit mit den Nebenwirkungen, welche anlässlich der intravenösen Injektion von Procain und Procainamid beschrieben sind auf (9). Andererseits erhielten wir seit der ersten Veröffentlichung der Reaktion vier Mitteilungen von Ärzten, die ganz ähnliche Symptome nach der Injektion von procainfreiem Antihistamin-Penicillin feststellten (Tab. III). Damit fällt das Procain als alleinige Ursache ausser Betracht.

Vergleicht man die bisher über akute, nichtallergische Procain-Penicillin-Nebenreaktionen unabhängig voneinander erschienenen Arbeiten, so stehen bei unseren Beobachtungen die neurologischen Symptome im Mittelpunkt, während die andern drei Gruppen besonders psychische Veränderungen mit Halluzinationen beschreiben. Handelt es sich um die gleiche Reaktion, hat bei der Reaktionsweise die Grundkrankheit eine Bedeutung, oder sind die Abweichungen lediglich auf Verschiedenheiten der Beobachter zurückzuführen? Die Auswahl des Krankengutes ist zweifellos unterschiedlich, indem die 8 Patienten von Batchelor und Mitarbeitern, die 3 Fälle von Randazzo und 7 der 9 Fälle von Björnberg an einer Lues litten, während sich unter den eigenen 24 Patienten nur ein einziger Lueskranker befindet. Die Frage der

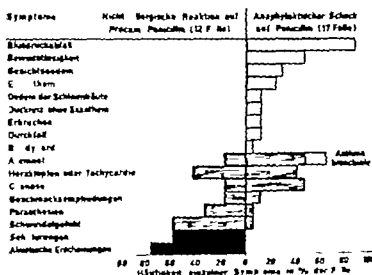


Fig. 1

Dieses Beispiel einer allergischen Allgemeinreaktion zeigt, dass selbst unter gleichzeitiger Verabreichung von Penicillin und Antihistamin das Antihistaminicum nicht in der Lage war die allergische Reaktion zu unterdrücken eine günstige Beeinflussung des Verlaufs der Reaktion durch Antihistamin ist trotzdem nicht ausgeschlossen.

Nicht allergische Reaktion

Im Gegensatz zu den akuten, allergischen Reaktionen sind in Tabelle II die Symptome der nichtallergischen Reaktionsform auf Procain Penicillin-Präparate zusammengefasst Es handelt sich um 24 eigene Beobachtungen Die Symptome betreffen besonders die Sinnesorgane mit akustischen Erscheinungen Schwindelgefühl Sehstörungen Geschmacksempfindungen und Paraesthesien. Engigkeitsgefühl Atemnot, Todesangst, Verwirrungszustand, manchmal Herzklopfen gehören zum klinischen Bild und erschweren die Abgrenzung von anaphylaktischen Reaktionen Erst nach sorgfältiger Ermittlung der klinischen Symptome, der Hautteste und serologischen Untersuchungen, welche sich im ganzen gesehen anders als beim anaphylaktischen Schock verhielten wurde bei der Hälfte der Fälle die Procain-Penicillinbehandlung fortgesetzt. Als auch unter Wiederholung der therapeutischen Dosen akute, allergische Nebenreaktionen ausblieben war die allergische Pathogenese so gut wie ausgeschlossen

Die nichtallergische Reaktionsform zeigt meist folgenden Verlauf Die Symptome stellen sich während oder nur 1—2 Minuten nach der intramuskularen Injektion von Procain-Penicillin ein. Sie sind zu Beginn der Reaktion am stärksten und bilden sich in 5 Minuten manchmal erst in einer viertel oder halben Stunde zurück. Vereinzelte Fälle weisen ein wellenförmiges An und Abschwellen der Erscheinungen wie bei anaphylaktischen Reaktionen auf Die Reaktionen ereigneten sich trotz allen Vorsichtsmassnahmen zur Vermeidung einer akzidentellen, intravaskularen Injektion. Selbst die strenge Beachtung der durch von Hochstetter (7) angegebenen Injektionstechnik in die ventrale Glutealmuskulatur liess die

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Polycythaemia Vera and Essential Thrombocythaemia

Two Variants of the Myelo-Proliferative Syndrome

By

Sven Åke Forsberg

Large series of polycythaemia vera cases (5 18) clearly show that the poly cythaemia is merely part of a panmyeloid proliferation, and is almost always associated with leucocytosis and/or thrombocytosis. Unlike the latter conditions, polycythaemia causes changes in the outward appearance, probably explaining why most interest at first was attached to this component of the disease. The name polycythaemia was adopted for what later proved to be a panmyelo-prolifera tive syndrome. It is known that this syndrome — sometimes here called the myelo-proliferative syndrome — can exist without polycythaemia, even in untreated cases. When polycythaemia has ppeared in the later course of the disease, this has sometimes been named masked polycythaemia (21) The disease which is characterised by a chroni cally raised thrombocyt count, sometimes t extreme levels, and with no known aetiology has usually been de scribed as essential" primary or haemorrhagic thrombocythaemia (3 7 8, 9 11 12, 12 a, 13 17 19 20 21 22 23 25 26, 32, 34 35 39 44) occa sionally megakaryocytic leukaemia

For the sake of simplicity the term primary (prim.) thrombocythaemia is used in the following and includes the two other terms essential and haemor rhagic unless otherwise stated.

We have had the opportunity to diagnose no less than six cases of myelo-proliferative syndrome, without initial polycythaemia, during a period of about two years. This has led us to believe that the incidence of such cases is higher than one would gather from the litera ture. These six cases will be presented and the clinical findings will be discussed in the light of earlier publications in this field.

Methods and normal values

Haemoglobin determinations prior to Sept 1958 were carried out using Autenrieth-Königsberger haemoglobinometer and 1 N HCl as diluting fluid. After Sept. 1958 the Beckman spectrophotometer was employed with ammonia as diluent. In both cases the Hgb standard was that of Enghoff 100 % Hgb = 15.3 g Hgb per 100 ml blood. With the latter method, Mårtensson at Falu Lasarett determined Hgb and serum iron in a series of healthy individuals. Results were as follows

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of cardiac failure, it should be noted that at the time for the blood volume determination of case 2, the patient had cardiac symptoms but had not been in failure since about two years.

The two remaining cases (nos 1 6) in which the TBV was not increased were in an anaemic phase at the time these tests were carried out. Case 1 had a low RCV and PV some months following treatment with ³²P Case 6 had a low RCV but a high PV and therefore normal TBV prior to ³²P treatment this co-incided with a period of melaena, with a severe anaemia and low haematocrit. v Porat (28) quotes Gibson et al. in pointing out that when anaemia is the result of bleeding there is often a lowering of the TBV In other words, the increase in PV does not usually compensate entirely for the drop in RCV

Polycythaemia vera is characterised by an increased RCV and increased TBV but often a lowered PV The more pronounced the polycythaemia, the more usual it is to discover lowered PV (5 18)

White cell count and differential count

In tw cases (nos. 3 6) a pronounced chronic leucocytosis was present, three patients (nos. 1 2 4) showed slight leucocytosis on sporadic occasions. In five (nos. 2, 3 4 5, 6) the differential count showed a relative neutrophilia with a tendency to a shift to the left

The incidence of leucocytosis in polycythaemia vera is given as about 70 % (5 18) Leucocytosis here is the result of an increase n neutrophil cells. Those cases described in the literature as "prim." thrombocythaemia have with few exceptions (13, 26) shown a concurrent tendency to leucocytosis.

Thrombocytes

All six patients had a thrombocytosis, which was extreme in case 6 and of a moderate degree in the others.

In polycythaemia vera the incidence of thrombocytosis is said to be 40—65 % (5 18) Published cases of "prim. thrombocythaemia have with few exceptions (13) shown extreme thrombocytosis (over 1 million/mm³) at some stage of the disease. But great variations are seen in the individual case and the thrombocyte count may even be normal at times (7 11 13 17)

Sternal marrow smears

In five cases (nos 2 3 4 5 6) the sternal marrow showed a generalised marked increase in cell numbers In three cases (nos. 4 5 6) there was a definite increase in the number of megakaryocytes.

The myelo-proliferation seen in polycythaemia is morphologically of a quantitative nature, which diminishes the value of sternal marrow smears as an a d in differential diagnosis. The typical finding is a generalised increase of nucleated marrow cells (18) In prim." thrombocythaemia, it is usual to find an increase in megakaryocytes, as well as a brisk erythropoiesis and myelopoiesis (8, 12 a, 31 44)

The spleen

Four cases had significant degree of splenomegaly (nos. 2, 3 4 5) This is frequently a clinical feature of polycythaemia vera (50 % —90 %) (5 18) Fanger et al. (8) collate 28 cases of thrombocythaemia in different diseases most of them myelo-proliferative diseases according to Dameshek (6) 23 of these 28 had splenomegaly Hardisty and Wolff (15) summarise 18 previously

Haemoglobin.

Men (50 cases) Age 19—60 (Mean 39.5) (Mean ± 2 standard deviations) = = 92 ± 5 %

Women (48 cases) Age 19—51 (Mean 36.0) (Mean ± 2 standard deviations) = 85.5 ± 11 %

Serum iron

Men (50 cases) (Mean ± 2 standard deviations) = 104 ± 54 γ %

Women (50 cases) (Mean ± 2 standard deviations) = 87 ± 62 γ %

In counting the thrombocytes we used Kristenson's method modified according to Ellerman's system (4). The skin of the finger tip was cleaned with ether, however, in place of olive oil-ether. In 20 healthy adults of both sexes, mostly men, duplicate thrombocyte counts were carried out. One blood specimen was drawn from each hand. Results:

Thrombocytes: Mean ± 2 standard deviations = 199 000 ± 48 000/mm³. The error of the method in a single analysis (2 standard deviations) = ± 12 000. The difference between duplicate determinations (2 standard deviations) = ± 17 000.

The blood volume was estimated by the Evans blue dye (T 1824) method. Normal control values were obtained by determining the patient's surface area with a nomogram according to Du Bois, and then estimating the normal value for total blood volume with the aid of Levinson and McFate's nomogram.

Clinical material

In this section a brief account is given of the clinical histories of our cases, together with their characteristic haematological findings (tables I—VI). Table VII shows the result of the blood volume determinations.

Discussion and comments

Haemoglobin, red cell count, and serum iron

None of our six patients showed polycythaemia initially and all of them had low serum iron prior to treatment with iron or ³²P. Three patients (nos. 3, 4, 6) were obviously anaemic. Normalisation of the serum iron was not achieved in any case by treatment with iron. A tendency to sideropenia has previously been described in polycythaemia vera (10, 30). This is probably the manifestation of depletion of the iron depots, following the synthesis of the pathologically high amount of haemoglobin. This depletion of iron is further accentuated in the event of haemorrhage. A tendency to polycythaemia was noted in five cases (nos. 1, 2, 3, 4, 5) following iron administration, which suggests that iron deficiency may inhibit the development of polycythaemia. This has been demonstrated earlier (1, 44) and regarded by others as dubious (10). The sideropenia of polycythaemia vera tends to disappear after treatment with ³²P (10, 30). This is illustrated in case 1 of our series. It seems reasonable to explain this by diminished erythropoiesis, and in turn a decrease in red-cell iron turnover. It is not unusual in "prim. thrombocythaemia, to find a slight polycythaemia or an anaemia, the latter often associated with a bleeding tendency.

Blood volume

The results of blood volume estimations and their timing in relation to the course of the disease are given in table VII in conjunction with tables I—VI.

In four cases (nos. 2, 3, 4, 5) the total blood volume (TBV) was pathologically increased, although the polycythaemia was of a moderate degree, judging from the red cell count. All four had a raised red cell volume (RCV) and three cases (nos. 2, 3, 4) also showed an increased plasma volume (PV). As the blood volume could be increased during periods

sis (14 40) 5 Proconvertin deficiency (29 37) 6. Prothrombin deficiency (22 37) 7 Pro-accelerin deficiency (22, 37) 8. Slowing-down of thrombocyte agglutination (36) 9 Accelerated clot retraction time (37) 10 Indication of the existence of some anti-coagulant substance in the blood-stream (25)

Various conclusions, some even contradictory have been reached by the different authors (42) It has been maintained that a haemorrhagic tendency is always associated with a high thrombocyte count (35) This is not supported by our findings, where in four of the patients there was a bleeding tendency despite a moderate thrombocythaemia. By no means all cases with high thrombocyte counts show a tendency to haemorrhage (11) Soulier et al. have analysed a number of different coagulation factors in a large series of thrombocythaemia (27 cases) They are in doubt as to whether the coagulation defects demonstrated in vitro are of any great significance in the aetiology of the haemorrhagic condition.

Treatment

Three patients (nos. 1 2, 6) were treated with ^{32}P The dosage, times of administration and the results, are shown in tables I—VI together with the clinical histories.

^{32}P has proved to be the most satisfactory agent to date in the treatment not only of polycythaemia vera (5 18) but also of prim. thrombocythaemia (9 33, 41 44) In polycythaemia vera ^{32}P seems to inhibit the leucocytosis or the thrombocytosis more readily than the polycythaemia (18) Bleeding in thrombocythaemia usually ceases after treatment with ^{32}P while the thrombocytosis diminishes or ceases concurrently (9 34 41 44) Thus one sees the apparent paradox that grave anaemia can in certain cases best be treated by an agent which inhibits erythropoiesis, as illustrated by our case 6 Recurrence of thrombocythaemia after treatment with ^{32}P is seen quite commonly but may respond to further treatment (41) Lüdin (20) and Gunz (12 a) have described cases of primary thrombocythaemia which responded well to treatment with myleran (busulphan)

Terminology

Dameshek (5 a, 6) is responsible for the unitary concept of the myelo-proliferative diseases. Polycythaemia vera and prim." thrombocythaemia are diseases with a panmyelo-proliferative tendency In the former condition the polycythaemia dominates the picture, while in the latter group it is the thrombocythaemia. Isolated primary thrombocythaemia without periods of leucocytosis or polycythaemia is rarely seen (13 26) It can be doubted if any such pure cases of thrombocythaemia really exist with long and close observation of the patients. Several authors (17 19 20 26 34 39) place prim. thrombocythaemia in a special disease category with a close resemblance to polycythaemia vera. Gunz (12 a) suggests that haemorrhagic thrombocythaemia should be classed alone. This appears illogical since it necessitates building a separate group of non haemorrhagic, primary thrombocythaemia Mallarmé and Anzepy (21) however maintained in discussing the classification of thrombocythaemias, that nothing in the clinical signs and symptoms of prim. thrombocythaemia justify their constituting a separate category These contradictory ideas are probably due to differences in the interpretation

published cases, and describe their own 5 cases of haemorrhagic thrombocythaemia. 7 of these 23 showed splenomegaly 8 of these 23 had undergone surgical removal of a pathologically altered spleen, three had splenic atrophy and five had no demonstrable splenic enlargement A number of other publications on "prim. thrombocythaemia indicate the high incidence of splenomegaly (3 7 8 12 a 13 17 19 20 35 39) but splenic atrophy or cirrhosis is very rare (13 41 a) In the group of "prim thrombocythaemia which is seen after splenectomy the spleen is always pathologically changed and thus provides the indication for operation In some of these cases the thrombocyte count was already raised before splenectomy (21) The patho-physiological relationship between splenomegaly and splenic atrophy on the one hand, and myelo-proliferation on the other has not yet been made clear Major surgical operations are often followed by transient thrombocytosis, which is said to be more pronounced after splenectomy than after other surgical procedures (33)

Symptomatology

Thrombo-embolic complications were seen in two patients (nos. 1 3) and possibly in a third (no 2) who had epilepsy with an E E. G focus, which might be ascribed to a cerebro-vascular insult A bleeding tendency was demonstrated in five cases (nos 2 3 4 5 6) In two cases (nos. 3 5) there was ulcus duodeni which necessitated partial gastrectomy It is not known what phase of myelo-proliferative disease patient no 5 was in, at the time of gastric resection

The most serious complications of polycythaemia vera are thrombo-embolism haemorrhage, and the development of myeloid leukaemia. The former two complications are also a threat to patients with "prim. thrombocythaemia, but it is not known how often these patients develop myeloid leukaemia.

At least two authors (20 35) have described thrombocythaemia with concurrent epilepsy which has disappeared after adequate treatment of the thrombocythaemia. Kupfer et al. (17) found at post mortem examination of a case of essential thrombocythaemia that brain section revealed numerous small blood vessels occluded by thrombocytes. There was no large infarct but widespread small areas of malacia and gliosis. Kissel et al and Olivarius (16 27) also report neurological complications of thrombocythaemia, mainly in the form of hemiparesis. Shaw and Oliver (34) report cases of thrombo-embolic episodes in the extremities in connection with thrombocythaemia. Thrombocythaemia is associated with an increased tendency to portal vein thrombosis, which may in turn give rise to bleeding oesophageal varices (11) In polycythaemia vera, as well as in "prim. thrombocythaemia, there appears to be an increased clinical incidence of duodenal ulcer (18, 21)

Haemorrhage is noted in 30 % of cases of polycythaemia vera (5) In haemorrhagic thrombocythaemia it is a condition of diagnosis, but even in cases named primary or essential thrombocythaemia haemorrhage is a common complication (7 8, 11 17 20 26) Many attempts have been made to discover the relationship between thrombocythaemia and the bleeding tendency One or more of the following disturbances of the coagulation mechanism have been noted

1 Inadequate thromboplastin function (1 15 34 37 38) 2 Fibrinogen deficiency (2) 3 Increased fibrinolysis (26) 4 Increased inhibition of fibrinoly

Table I. Characteristic haematological finding and therapy in case I

Year	Month	Hgb %	Red cell count millions per mm³	Hematocrit %	Reticulocyte count %	White cell count per mm	Thrombocyte count thousands per mm	Serum iron γ per 100 ml	Transferrin γ per 100 ml	Sedim. rate mm per hour	Therapy
1959	II	83	4.2	44	0.3	9,000	730				
	III	86	4.2	43	0.6	12,800	640	28	433	4	Iron per os started
	IV	86	4.4				550			1	
	VII	113	4.5		1.8	10,000	450			2	Iron stopped
	VIII	114	5.5	57	3.3	11 000	530	30		2	
	IX	103	5.1		1.8	10,400	730				32P 6 mC
	XII	68	3.3	37	2.2	4,600	200	159	270	32	
	XII	59	2.9				280	122		36	
1960	II	66	3.2			3,600	190			20	
	VIII	53	2.8	30	0.7	6,100	390	33	363	40	
	IX										Iron per os started
	XII	59	3.0			5,400	430			19	
1961	III	63	3.3			6 400	380	23		24	Iron stopped

smears in March, July Aug., 1959 and July 1960 showed no abnormal features. *Straight X-ray of abdomen* in March, 1959 revealed no splenomegaly. *Blood volume*, see table VII. Among other tests with normal results were coagulation time, bleeding time, liver function tests, serum electrophoresis, fibrinogen.

Case 2. Female born 1905. This patient has had marked hypertension, diastolic pressure around 100—130, and has not responded well to treatment. Finally towards the end of 1960, the blood pressure was successfully lowered with Ismelin and Esidrex. Since 1956 there has been evidence of cardiac insufficiency and at the beginning of 58 she was in failure. During the first half of '60 she had several episodes of tachycardia-arrhythmia and fall in blood pressure. She had chronic pyelonephritis with tendency to proteinuria, bacteriuria and pyuria, episodes of acute urinary tract infection, and progressing renal insufficiency with slightly raised blood non-protein nitrogen, dating from 1960. Between Dec., '57 and the end of '59 the patient showed tendency to easy bruising, and once, possibly twice she had haemarthrosis, as well as menorrhagia, and para-pharyngeal haematoma. The haematological picture was characterised by a slight tendency to leucocytosis and sideropenia. Iron was given by mouth during autumn, 1959 and during this time the patient developed slight polycythaemia and moderate thrombocythaemia. I Sept. 1959 we gave 32P. After this, the leucocytosis and thrombocythaemia disappeared, while the polycythaemia persisted. Since the end of 59 she has had no further haemorrhagic symptoms. Between Jan. 59 and May '60 attacks of epileptic "grand mal" occurred. E. E. G. revealed unilateral focal changes which have almost completely regressed. In association with the first attack there was papilloedema in both fundi, without any alteration in the blood pressure.

Summary of laboratory findings. Most of the haematological findings are shown in table II. *Differential count* was done on 15 occasions, and indicated relative preponderance of neutrophils, with a slight shift to the left. *Sternal marrow smears* in Aug. and Sept., 1959

of terms rather than divergent opinions on the panmyelo-proliferative nature of the two diseases. If cases of panmyelo-proliferative syndrome in which polycythaemia predominates, are to be called "polycythaemia vera" it is equally justifiable that those in which thrombocythaemia dominates a similar syndrome might be known as "primary thrombocythaemia". There is no qualitative difference between the two conditions either in the pathological anatomical or in the clinical picture. The sum total of the evidence presented here suggests that both conditions are merely variations of the same type of disturbance of function of the bone marrow. The simplest solution, therefore, to the problem of terminology is to resort to the most central and fundamental feature in the pathological process, which is myelo-proliferation and to adopt the term "myelo-proliferative syndrome". This can be further qualified by describing the peripheral blood picture which is a secondary event. Nothing however is thereby stated about the aetiology of the bone marrow dysfunction. In their proposal for the aetiological sub-division of thrombocythaemias Mallarmé and Anzepy (21) devoted a special category to thrombocythaemias observed after splenectomy. It is doubtful whether this provides a sound basis for categorisation, since the possible aetiological significance of the spleen remains unclear, particularly moreover since a number of patients show thrombocytosis prior to splenectomy.

The diagnosis in cases such as the six reported here, does not present itself too readily. When investigating such cases in the future it would be of great interest to measure the blood volume systematically before and after iron administration and after treatment with ^{32}P. It is possible that estimation of the alkaline *phosphatase activity in the neutrophil* leucocytes might also be of diagnostic value. It is still uncertain what is the most suitable point in the course of the disease for the instigation of ^{32}P treatment, since there is as yet no radical cure for the condition. We have decided, however, to administer ^{32}P even to patients nos. 3 and 4 in our series.

Case 1. Female, born 1901. As long as we have observed this patient she has had a mild hypertension, without heart involvement. Renal function tests have revealed some impairment of concentration, and a slightly lowered endogenous creatinine clearance. In the summer of 1958 she began to have impaired circulation in the toes of both feet. The entire observation time may be divided into 3 periods.

1st period prior to the commencement of iron therapy in March, 1959. This was characterised by stationary symptoms in the toes, a moderate thrombocytosis, slight leucocytosis, and sideropenia.

2nd period comprises the time from the start of iron treatment to the start of ^{32}P in Sept., 1959. During the last month of iron therapy the circulation in the toes deteriorated. A necrotic ulcer appeared, but gradually healed completely. The patient was put on continuous dicumarol therapy. A mild polycythaemia developed. The haematological picture remained otherwise unchanged. ^{32}P was given.

3rd period comprises the time following ^{32}P treatment. She then developed a moderate anaemia, while the leucocytosis, thrombocythaemia, and sideropenia disappeared. There was a striking improvement in the toe symptoms. About a year after her course of ^{32}P she again displayed sideropenia, and a slight thrombocythaemia. The circulation in the toes deteriorated once more. Small ulcers made their appearance. There has not been any sign of bleeding.

Summary of laboratory findings. Most of the haematological findings in this case are shown in table I. *Differential count* done on 6 occasions, was normal each time. *Sternal marrow*

Table I. Characteristic haematological findings and therapy in case 1

Year	Month	Hgb %	Red cell count millions per mm	Haematocrit %	Reticulocyte count %	White cell count per mm³	Thrombocyt. count thousands per mm	Serum Iron γ per 100 ml	Transferrin γ per 100 ml	Sedim. rate mm per hour	Therapy
1959	II	83	4.2	44	0.3	9,000	730				
	III	86	4.2	43	0.6	12,800	640	28	453	4	Iron per os started
	IV	86	4.4				550			1	
	VII	113	5.5		1.8	10,000	450			2	Iron stopped
	VIII	114	5.5	57	3.3	11 000	550	30		2	
	IX	103	5.1		1.8	10,400	730				³²P 8 mC
	XII	68	3.3	37	2.2	4 600	200	159	270	32	
	XII	59	2.9				200	122		36	
1960	II	66	3.2			5,600	190			20	
	VIII	55	2.8	50	0.7	6,100	390	33	563	40	
	IX										Iron per os started
	XII	59	3.0			8 400	450			19	
1961	III	63	3.3			6 400	580	23		24	Iron stopped

smears in March, July Aug., 1959 and July 1960 showed no abnormal features. *Straight X-ray of abdomen* in March, 1959, revealed no splenomegaly. *Blood volume* see table VII. Among other tests with normal results were coagulation time, bleeding time, liver function tests, serum electrophoresis, fibrinogen.

Case 2. Female, born 1905. This patient has had marked hypertension, diastolic pressure around 100—130, and has not responded well to treatment. Finally towards the end of 1960, the blood pressure was successfully lowered with Ismelin and Esidrex. Since 1956 there has been evidence of cardiac insufficiency and at the beginning of '58 she was in failure. During the first half of '60 she had several episodes of tachycardia, arrhythmia and fall in blood pressure. She had chronic pyelonephritis with tendency to proteinuria, bacteriuria and pyuria, episodes of acute urinary tract infection, and progressing renal insufficiency with slightly raised blood non-protein nitrogen, dating from 1960. Between Dec. '57 and the end of '59 the patient showed tendency to easy bruising, and once, possibly twice, she had haemarthrosis, as well as menorrhagia, and para-pharyngeal haematoma. The haematological picture was characterised by a slight tendency to leucocytosis and sideropenia. Iron was given by mouth during autumn, 1959 and during this time the patient developed a slight polycythaemia and moderate thrombocythaemia. In Sept. 1959 was gave ³²P. After this, the leucocytosis and thrombocythaemia disappeared, while the polycythaemia persisted. Since the end of '59 she has had no further haemorrhagic symptoms. Between Jan. '59 and May '60 attacks of epileptic "grand mal" occurred. E. E. G. revealed unilateral focal changes which have almost completely regressed. In association with the first attack there was papilloedema in both fundi, without any alteration in the blood pressure.

Summary of laboratory findings. Most of the haematological findings are shown in table II. *Differential count* was done on 15 occasions, and indicated relative preponderance of neutrophils, with a slight shift to the left. *Sternal marrow smears* in Aug. and Sept., 1959

Table II Characteristic haematological findings and therapy in case 2

Year	Month	Hgb %	Red cell count millions per mm³	Hematocrit %	Reticulocyte count %	White cell count per mm³	Thrombocyte count thousands per mm³	Serum Iron γ per 100 ml	Transferrin γ per 100 ml	Sedim. rate mm per hour	Therapy
1957	XII	75	4 0			11,600				1	
1958	II	79	4.1			10,800				2	
	VI	69	3 6			8,400				4	
	VI	78	4 1			7,800					
	XII	87	4.5			14,800	370			1	
1959	IV	84				11 600				1	
	VIII	79	4.2			8,300	280	24		1	Iron per os started
	VIII	89	5.9	51	2.1	9 400	600	40	376		
	IX	93	6.0		3.5	9,300	380				Iron stopped. ³²P 6 mC
	XI	90	5.2	55	2 0	4,900	720	27		1	
1960	I	100	5 1	57		7 400	200			3	
	II	100	5.3	55		4,800	190				³²P 5 mC
	IV	103	5.6			5,600	190			1	
	VII	96				6,200				1	
	XI	107	5 1	52	1.9	5,200	180	51		1	
1961	II	98	5 1			8,400				2	

showed a highly cellular but normal marrow *Straight X-ray of abdomen* in Aug 59 revealed a moderate degree of splenic enlargement. *Blood volume* see table VII *Bleeding time* was slightly prolonged in Nov 59 though normal on several occasions previously *Fibrinogen* in Aug 59—0.6 g Nov 59—0 3 g %. Other investigations with normal findings liver function tests, serum electrophoresis, clotting time.

Case 3 Female, born 1886 A duodenal ulcer was discovered in this patient in June 1959 There was gastric retention, positive Weber in the faeces, moderate anaemia sideropenia and a leucocytosis. During the iron therapy which followed, she developed polycythaemia the leucocytosis persisted, and a moderate thrombocythaemia was also noted In Feb. '60 a partial gastrectomy was performed and there has been no further bleeding The haematological picture remained virtually unchanged In May '60 there were signs of arterial insufficiency in the right leg, and angiography showed a marked narrowing of the right femoral artery Permanent dicumarol treatment was instigated. There have been no abnormal clinical signs in the heart or urinary tract, but lung examination revealed slight emphysema.

Summary of laboratory findings Most of the haematological findings are to be seen in table III *Differential count* performed on 4 occasions showed at times a relative neutrophilia. *Sternal marrow smears* in June and Oct 1960 showed highly cellular normal marrow *Straight X-ray of abdomen* in Oct 60 showed a normal-sized spleen *Blood volume* see table VII *Fibrinogen* during 1960 Jan.—0 64 g June—0.62 g Oct —0.67 g Other tests which gave normal results bleeding time, clotting time liver function tests, serum electrophoresis.

Table III. Characteristic haematological findings and therapy in case 3

Year	Month	Hgb %	Red cell count millions per mm³	Haematocrit %	Reticulocyte count %	White cell count per mm³	Thrombocyte count thousands per mm³	Serum iron γ per 100 ml	Sedim. rate mm per hour	Therapy
1959	VI	65	4.1		5.4	19,800		9	4	
	VII	77	4.4			10,800		74	2	Iron per os started
	IX	93				15,100			2	Iron stopped
1960	I	95	6.1	58	4.5	22,900	490	40	1	
	I	82	5.3	54	2.1	10,000	510		0	
	II	75								Ventricle resection
	IV	73						17	3	
	V	73				12,000	330	44	1	Imferon, total 1.2 g
	VI	80	4.3	56	2.6	17,200	650	15	1	
	X	86	4.3	53	6.9	11,000	500			
	XI	83	4.8		1.5	19,000	560	20		
1961	IV	72	7.2			24,800			2	

Table IV. Characteristic haematological findings and therapy in case 4

Year	Month	Hgb	Red cell count millions per mm³	Haematocrit %	Reticulocyte count %	White cell count per mm³	Thrombocyte count thousands per mm³	Serum iron γ per 100 ml	Transferrin γ per 100 ml	Sedim. rate mm per hour	Therapy
1959	IX	42	3.3		5.1	8,000	430	10		7	Iron per os started
	IX	64	3.3		2.9	4,800	520			6	
	X	83	4.4			13,000	400	21		5	
	XI	84									
1960	I										Strumectomia
	IX	81	6.6		0.8	14,200	550				Iron stopped
	XI	83	4.5	50	2.4	8,800	320			1	
	XII	77	5.1	52	1.2	16,800	640	30	387	2	
1961	VI	68		44			940		360		

Table V Characteristic haematological findings and therapy in case 5

Year	Month	Hgb %	Red cell count millions per mm³	Hematocrit %	Reticulocyte count %	White cell count per mm³	Thrombocyte count thousands per mm³	Serum iron γ per 100 ml	Transferrin γ per 100 ml	Sedim. rat mm per hour	Therapy
1955	I	92				15,200				2	
1960	VII	78	4.9			8,600	320			6	
	VIII	84	4.3	46	3 0	6,200	400	43	400	2	Iron per os started
	X	105	5.3	64	1.2	6 000	200	74		1	Iron stopped
1961	II	110	5 1			7,200					

Case 4 Female, born 1903 This patient had from her youth a slow-growing, atoxic, nodular goitre. From the middle of the 1950's she had noticed a tendency to easy bruising, and prolonged bleeding after small traumata, such as venepuncture. Partial thyroidectomy in Nov 59 was complicated post-operatively by a severe haemorrhage.

In Sept. 1959 a hypochromic, iron-deficiency anaemia was discovered, together with a moderate thrombocythaemia and splenomegaly After some months iron per os the Hgb became normal. In Nov '60 she was found to have slight polycythaemia, a tendency to leucocytosis, and persistent thrombocythaemia. She had assumed a more highly coloured appearance. The spleen showed the same degree of enlargement. Examination of heart, lungs, and urinary tract consistently revealed normal findings.

Summary of laboratory findings Most of the haematological findings appear in table IV Differential count on 3 different occasions showed a relative neutrophilia, once there was a shift to the left. *Sternal marrow smears* in Sept. 1959 showed a highly cellular marrow with a moderate increase in the number of megakaryocytes. *Straight X-ray of abdomen* in Sept. 59 confirmed a significant splenomegaly *Blood volume* see table VII

Other investigations which proved normal bleeding time, clotting time liver function tests, serum electrophoresis, fibrinogen, Coombs test, haptoglobin, prothrombin index.

Case 5. Male, born 1901 Early in the 1950's this patient had several episodes of haemorrhage from a peptic ulcer Partial gastrectomy was performed in 1956. Between autumn 59 and autumn 60 he had on several occasions enormous haematomata in the extremities, following insignificant injuries. In July 1960 he was found to have an iron deficiency with a moderate thrombocytosis. During the course of oral iron which followed, he developed polycythaemia. Splenomegaly was also noted Heart, lung and urinary findings were normal.

Summary of laboratory findings. Most of the haematological findings are shown in table V *Differential count* on ? occasions showed a relative neutrophilia and a pronounced anisopoikilocytosis. *Sternal marrow smears* in Oct. '60 gave a highly cellular marrow with a moderate increase in the number of megakaryocytes. *Straight X-ray of abdomen* in July 1960 revealed splenomegaly *Blood volume* see table VII *Fibrinogen* during 1960 Aug —0.56 g% Oct —0 47 g% Other tests which proved normal included bleeding time clotting time Prothrombin index, liver function tests, and serum electrophoresis.

Table VI Characteristic haematological findings and therapy in case 6

Year	Month	Hgb %	Red cell count millions per mm³	Reticulocyte count %	White cell count per mm³	Thrombocyte count thousands per mm³	Serum iron γ per 100 ml	Transferrin γ per 100 ml	Sedim. rate mm per hour	Therapy
1959	VI	28	1.7	7.8	14,800	1 400	23		4	Iron per os
	VI	51	3 0		12,300		10			
	VII	54	3.5	1 7	18,600					Iron parenterally
1960	III	58	3 2	7.0	12,100	1,250	32		2	Iron parenterally
	III	55	3 7	4.1	17 400	1,680				
1961	III	29	1 4	2.1	13,200	1,200	7	579	2	Iron parenterally
	IV	37	2.5	8 7	8,400	3,400				³²P 5 mC
	VI	66	3 6		3,100	460			2	

Table VII Results of blood volume estimations in patients 1—6

The results in case 4 are somewhat uncertain. Three estimations were made but one of these was complete failure. In the other two, the injected volume of Evans blue dye was 9 ml instead of 10 ml. Taking these facts into account, the results obtained seem to suggest pathological rise in blood volume on both occasions.

Case no	Date of investigation	Body weight kg	Body surface area m²	Haemato-crit	TBV ml	TBV normal value ml	PV ml	RCV ml
1	4. 12. 1959	58	1 58	33	3,010	4,100	2 000	1,010
2	19 1 1960	58	1.69	37	7 140	4,250	3,240	3,900
3	28. 10. 1960	43	1 42	55	6,960	4 050	3 416	3,544
4	6. 12 1960	64.5	1 70	52	7,560	4,200	3,780	3,780
5	20. 10 1960	65	1 88	61	6,940	5,600	2,870	4 070
6	19 4 1961	46 1	1 43	23	4,050	3,800	3 078	972

TBV total blood volume PV plasma volume RCV = red cell volume

Case 6. Female born 1885. This patient had, from 1916 at the latest, had an anaemia for which she had received iron and liver substances. In '56, '58, '59, '60 and '61 she had been admitted to hospital for melaena, the cause of which was never established, despite repeated X-ray examination. On her 3 latest admissions severe sideropenic anaemia was noted, as well as consistent leucocytosis and extreme thrombocythaemia. In April 1961 she received ³²P. About six weeks later the bleeding had stopped, Hgb was rising with the help of iron therapy, the leucocytes were now normal in number and there was only very slight thrombocythaemia. There were physical signs of aortic stenosis, and the heart was moderately enlarged. There was no sign of cardiac failure, however. Blood pressure was around normal. There was no sign of renal damage.

Summary of laboratory findings Most of the haematological findings are shown in table VI *Differential count* showed, on 3 occasions, a relative neutrophilia, and twice there was evidence of a shift to the left *Sternal marrow smears* in March 61 showed an intensive normoblastic erythropoiesis as well as active myelopoiesis and a marked increase in megakaryocytes. *Straight X ray of abdomen* in March, 1961 gave no evidence of splenic enlargement. *Blood volume* see table VII Other tests with normal results included Schilling test, serum B-12 haptoglobin, liver function tests, serum electrophoresis, and fibrinogen.

Summary

Polycythaemia vera has been shown to be part of a panmyeloproliferative syndrome. Chronic thrombocytosis of unknown aetiology is usually named primary essential or haemorrhagic thrombocythaemia. Six cases are presented and discussed with reference to earlier publications. Normal values for Hgb serum-iron and platelet count are given from our own laboratory Blood volume determinations have been made in all patients The discussion comprises the following titles Haemoglobin, red cell count and serum iron Blood volume White cell count and differential count Thrombocytes Sternal marrow smears The spleen Symptomatology Treatment Terminology All six patients had signs of a panmyeloproliferative syndrome with thrombocythaemia and were without polycythaemia from the beginning of the study During iron therapy five patients developed a slight polycythaemia. There was a tendency to increase of red cell volume and plasma volume at this time.

The author concurs with the opinion that there is no qualitative difference between polycythaemia vera and primary thrombocythaemia and that these two syndromes are only two variants of one disease. To emphasize the central point of the pathogenesis, the disease could very well be named a myeloproliferative syndrome with additional description of the peripheral blood picture that is a secondary event.

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Summary

Polycythaemia vera has been shown to be part of a panmyeloproliferative syndrome. Chronic thrombocytosis of unknown aetiology is usually named primary essential or haemorrhagic thrombocythaemia. Six cases are presented and discussed with reference to earlier publications. Normal values for Hgb serum-iron and platelet count are given from our own laboratory Blood volume determinations have been made in all patients. The discussion comprises the following titles Haemoglobin red cell count and serum iron Blood volume White cell count and differential count Thrombocytes Sternal marrow smears The spleen Symptomatology Treatment Terminology All six patients had signs of a panmyeloproliferative syndrome with thrombocythaemia and were without polycythaemia from the beginning of the study During iron therapy five patients developed a slight polycythaemia. There was a tendency to increase of red cell volume and plasma volume at this time.

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Haemophilia in Sweden

II. Carriers of Haemophilia A and B

By

Inga Marie Nilsson, Margareta Blombäck, Olof Ramgren and Irene v. Francken

During the past 50 years, numerous attempts have been made to detect the carrier state in haemophilia on the basis of abnormalities in the coagulation mechanism, but the results have often been contradictory. In a previous paper (12) the results of a study of 28 definite or possible carriers of haemophilia A were reported. The antihaemophilic factor (AHF or factor VIII) content was determined by a method based on the ability of the carrier's plasma in dilution to correct the recalcification time of haemophilia A plasma under proper standard conditions. Significantly low AHF values were found in all carriers of fertile age indicating that the gene of haemophilia A is semidominant and that it would be possible to trace carriers of haemophilia A. We have extended this investigation, and have now studied altogether 79 definite, probable or potential carriers of haemophilia A, and 41 definite probable or potential carriers of haemophilia B.

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Methods

AHF determinations. The plasma AHF was assayed on haemophilia A plasma (AHF content < 1 per cent) in a recalcification system, in which the ability of the citrated control plasma and the test plasma to correct the prolonged recalcification time of citrated haemophilia A plasma is compared. This method has been described and commented on in previous papers (11, 12). The plasma of the patients was assayed in dilutions of (1/20) 1/50 and 1/100. Normal values ranged from 60 to 160 per cent, with a mean of 100 ± 17.5 per cent.

The AHF content in a group of 10 healthy women past the menopause ranged from 76 to 199 per cent, with a mean of 159 per cent.

Haemophilia B factor (factor IX) determinations. The B factor content of the plasma samples was assayed on haemophilia B plasma in a recalcification system, in which the citrated control plasma and the test plasma

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Table I. Carriers

Family			Carrier state	Case		Bleeding Symptoms	Date of invest. Mth/Yr	AHF % of normal	B factor % of normal	Coagulation time min.	Prothrombin consumpt. %	Comments
No.	Haemophilia Type	Coordinate No.		Initials	Year of birth							
4	A severe	V:3	definite	U.H.N	1931	None	2/58	47	128	6	4	
8	B severe	IV 10	potential	L.C.	1927	None	2/59	79	67	9	2	
10	A severe	IV 10	potential	E.A.	1941	None	1/60	68	73	6	9	
11	A severe	III:5	definite	K.L.	1906	None	2/58	72	160	8	5	Meno-pause
14	B severe	IV:3	potential	G.G.	1927	None	11/58	90	23	13	38	
18	A severe	V 11	potential	K.W	1937	None	7/60	46	80	9	22	
							7 60	50	68	—	—	
19	A severe	IV:2	definite	L.T	1918	None	9 60	35	128	11	43	
22	A severe	V.8	definite	E.E.	1914	None	6/59	53	94	9	18	
22	A severe	VI 5	definite	L.K.	1938	Tooth extract.	6 59	28	103	15	20	
								27	—	—	—	
27	A moderate	IV 27	potential	B.S.	1925	None	8 56	60	100	4	—	
							10 56	55	—	11	9	
27	A moderate	IV 28	definite	U.K.	1932	None	10 56	40	100	7	8	
27	A moderate	V 16	potential	A.J	1941	None	1 61	43	—	11	13	
							1 61	46	93	11	—	
28	A severe	V 1	definite	L.W	1914	Haematoma	11 57	21	86	8	8	
							5 58	32	—	6	—	
							11 58	42	96	4	28	
28	A severe	VI 2	potential	A.J	1937	Haematoma	11 57	24	—	5	—	grav-
							3 60	63, 47	110	7	67	mens IV
							4 60	20	—	5	28	after abort mens
32	A severe	V 5	potential	A.B.T	1944	None	7 59	120	73	10	—	
							9 59	97	—	—	—	
32	A severe	V 7	potential	B.T	1918	None	7 59	50	75	6	—	
							9 59	52	—	—	—	
32	A severe	III,3	definite	M.C	1900	None	9 59	33	88	9	—	
32	A severe	III 4	potential	S.A.	1901	None	10 59	81	84	10	—	
32	A severe	III,5	definite	A.J	1903	None	9 58	54	114	12	—	
32	A severe	III 6	potential	R.L.	1905	None	10 59	42	78	7	—	
							12 59	40	—	—	—	
32	A severe	III 7	potential	M.A.	1908	None	3 60	35	93	10	76	

are compared for ability to correct the prolonged recalcification time of haemophilia B plasma. The plasma of the patients was assayed at dilutions of 1/50 and 1/100. Normal values ranged from 60 to 160 per cent.

The plasma content of haemophilia B factor was determined in 16 healthy women aged 51—77 years It was found to range from 55 to 150 per cent.

Coagulation time. This was determined by the method of Hedenius (9) but with the modification that the size of the tubes was 55 × 10 mm. The determinations were performed at room temperature. The normal values ranged from 6 to 12 minutes.

Prothrombin consumption test This test was performed as described by Biggs & Macfarlane (4)

Other coagulation factors These were determined as described earlier (11)

Clinical material

A *definite carrier* (cf. Bentley & Krivit 1960) is defined as

a. A woman who is a daughter of a haemophiliac,

b. A woman with two or more children with haemophilia and/or proved carrier state

c. A mother of a single haemophiliac or of a proved carrier offspring, and with other haemophilic relatives.

A *probable carrier* is defined as a woman with only one haemophilic son or one haemophilic daughter's son but no other relatives with haemophilia.

A *potential carrier* is defined as a woman without haemophilic sons, but with a 50 to 25 per cent genetic chance of being a carrier of haemophilia.

In a previous paper (12) the two latter groups were denoted as "possible carriers.

Carriers of haemophilia A The clinical material consisted of altogether 79 women belonging to 46 families in which haemophilia A had been diagnosed in at least one male member of each family (tables I and II According to the criteria listed above 33 of these women can be regarded as definite 14 as probable and 32 as potential carriers. Fifty-nine of the women studied were of fertile age, 6 had not reached the menarche and 14 were postmenopausal. Altogether 54 of the women investigated belonged to families with severe haemophilia A, 9 to families with moderate and 16 to families with mild haemophilia A.

Fourteen women complained of easy bruisability and increased bleeding tendency e.g. after tooth extraction. The others had had no bleeding symptoms. The women were otherwise healthy

Whenever possible, determinations were made on more than one occasion.

Carriers of haemophilia B The material consisted of 41 women, belonging to 18 families in which haemophilia B had been diagnosed (tables I and II) Altogether 18 of these women can be regarded as definite, 5 as probable and 18 as potential carriers. Thirty of the women studied were of fertile age, 3 had not reached the menarche and 8 were postmenopausal Altogether 27 of the women investigated belonged to families with severe haemophilia B, 5 to families with moderate and 9 to families with mild haemophilia B.

Seven women complained of ready bruisability and increased bleeding tendency e.g. after tooth extraction The others had no history of bleeding symptoms. Case IV 4, family 71 suffered from disseminated sclerosis, and was under treatment with corticosteroids. The other women tested were healthy

Results

The results of the coagulation data are recorded in tables I—II and figures 1—2

Carriers of haemophilia A Thirty-one of the 33 definite carriers had low AHF values, ranging from 15 to 60 per cent of normal (tables I and II fig 1) Two definite carriers had normal AHF values (case III 3 in family 11 and case II 6 in family 114) The first woman K. L. born in 1906 was over the menopause The other E. J born in 1934 was of fertile age and showed AHF values varying between 44 and 100 per cent Nine of the 14 probable carriers showed low AHF values, the 5 probable carriers with normal AHF values all being over the menopause

Table I Cont.

Family			Carrier state	Case		Bleeding Symptoms	Date of invest. Mth/Yr	AHF % of normal	B factor % of normal	Coagulation time min.	Prothrombin consumpt. %	Comments
No.	Haemophilia Type	Coordinate No.		Initials	Year of birth							
61	B severe	III 3	potential	B. S.	1918	Haematoma	11/58	68	43	6	6	
61	B severe	III 4	definite	D. A.	1920	None	12/57	50	56	8	—	
							6/58	—	54	4	—	
							11/58	85	33	7	14	
							4/60	73	50	10	21	
61	B severe	III 7	potential	K. B.	1930	None	12/57	90	72	9	9	
							6/58	—	45	6	—	
							11/58	104	55	6	—	
							4/60	116	88	9	—	
61	B severe	III 8	potential	L. B. J.	1933	None	12/57	98	45	8	9	
							11/58	75	38	6	3	
63	A severe	III 1	probable	R. A.	1899	None	1/57	100	—	10	31	
64	B mild	IV 2	definite	E. F.	1923	None	3/60	75	33	9	34	
65	A mild	III 7	definite	D. J.	1921	None	1/57	29	—	6	29	
68	A severe	IV 2	potential	D. J.	1932	None	3/60	83	65	6	—	
							4/60	110	100	6	11	
70	A severe	III 4	probable	L. M.	1893	None	10/57	133	90	10	—	
							12/57	148	120	4	3	
70	A severe	IV 2	probable	G. M.	1922	Haematoma + bleed after tooth extract.	10/57	30	—	4	0	
							12/57	40	80	6	17	
							5/58	36	—	6	—	
71	B mild	IV 4	definite	E. A.	1913	Haematoma + bleed after partus	11/58	71	54	8	2	
72	A severe	III 15	definite	B. B.	1919	Haematoma	10/57	36	90	5	7	
							11/57	33	—	—	11	
73	A severe	IV 12	definite	A. O.	1903	None	6/58	36	—	6	29	
73	A severe	IV 19	potential	I. C.	1934	None	9/59	48	150	13	27	
							9/59	43	—	—	—	
							11/59	23	—	10	12	
							4/61	33	—	—	—	
74	A severe	III 7	definite	I. K.	1922	None	12/59	54	73	13	40	
							1/60	55				
76	A severe	III 9	probable	M. S.	1912	None	11/57	31	70	8	5	

Table I Cont

Family			Carrier state	Case		Bleeding Symptoms	Date of Invest. Mth/Yr	AHF	B factor	Coagulation time min.	Prothrombin consumpt. %	Comments
No	Haemophilia Type	Coordinate No.		Initials	Year of birth			of normal	of normal			
32	A severe	III 8	potential	B. N	1911	None	10/59	90	70	10	–	
							12/59	88	–	–	–	
32	A severe	IV 5	definite	G T	1919	None	10/58	25	94	9	47	
							11/58	24	–	11	–	
							12/58	23	–	5	–	
32	A severe	IV 8	potential	I. E.	1935	None	9/59	49	106	7	–	
							3/60	55	–	11	15	
32	A severe	IV 11	definit	G S.	1926	None	3/59	35	95	10	18	
32	A severe	IV 7	potential	A. D	1930	None	9 59	93	120	10	–	
							3/60	70	–	9	23	
32	A severe	IV 13	potential	K. M.	1935	None	9/59	48	102	6	–	
							3/60	50	–	19	6	
32	A severe	IV 14	potential	Y. A.	1932	None	9 59	54	120	9	–	
							3 60	50	–	11	–	
36	A severe	IV 2	potential	B W	1929	None	5 59	113	135	10	29	
							5 59	79	110			
							12 59	110	–			
							3 60	102				
37	A severe	IV 2	definite	M S.	1929	None	7 57	62	–	5	–	pregnant TGT
							11 57	38	106	6	–	
							12 57	45		–		
							1 58	45		–		
							2 58	41	–	–	–	*
							2 58	69	–	–	–	
							3 58	40	–	–	–	partus
							11 58	28	–	8	–	
40	A severe	V 4	definit	K. H	1929	Non	4 60	17	76	7	14	
							5 60	31	–	6	–	
44	B severe	VI 11	definit	E. Ö	1924	None	2 60	121	43	6	5	
56	B severe	III 5	potential	S. A.	1894	None	7 60	118	135	8	18	
56	B severe	III 6	potential	A. H	1896	Non	7 60	107	155	9	11	
56	B severe	III 7	potential	E. T	1898	None	7 60	110	135	8	24	
56	B severe	III 8	definite	A. J	1900	None	10 57	80	133	6	0	
							12 57	80	90	–	–	
							5 60	–	120	8	33	
							7 60	134	118	9	37	
59	B severe	IV 9	definite	M. P	1927	None	3 60	70	35	9	17	
61	B severe	III 2	definite	L. K.	1917	None	12 57	74	42	8	5	
							6 58	–	50	7	–	
							11 58	125	40	7	6	

Table I Cont.

Family			Carrier state	Case		Bleeding Symptoms	Date of invest. Mth/Yr	AHF % of normal	B factor % of normal	Coagulation time min.	Prothrombin consumpt. %	Comments
No.	Haemophilia Type	Coordinate No.		Initials	Year of birth							
98	A mild	II:10	probable	E. A.	1906	None	3/57	86	—	7	12	
99	B severe	II:6	definite	K. G.	1914	None	3/60	70	43	10	28	
101	A mild	III:7	definite	H. V	1902	None	11/58	34	98	5	—	
104	A mild	IV:4	potential	S. S.	1930	None	1/59	118	85	8	17	
							1/59	103	—	8	—	
105	A severe	III:5	definite	R. B.	1928	None	1/58	42	66	11	28	
106	A severe	II:4	probable	S. J	1908	None	8/57	23	—	8	8	
108	A severe	II:6	probable	E. V	1912	None	11/57	46	100	5	0	
110	B severe	IV:9	definite	E. H.	1915	None	7/57	55	50	9	6	
							7/57	50	—	11	—	
							3/58	61	55	11	6	
							6/60	90	35	13	—	
							7/60	35	40	—	—	
111	B severe	III:6	definite	E. J	1905	None	11/57	85	52	10	11	
							6/58	84	59	11	21	
112	B severe	III:5	potential	E. F	1898	None	6/58	80	76	12	2	
112	B severe	IV:2	potential	H. P	1941	None	6/58	160	123	10	10	
114	A severe	II:2	potential	N. L.	1929	Tooth extract.	5/60	41	—	9	24	
							5/60	34	—	8	—	
114	A severe	II:6	definite	E. J.	1934	None	9/59	100	75	17	6	
							9/59	91	—	21	—	
							5/60	61	—	8	21	
							5/60	73	—	—	28	
							5/60	44	—	—	—	
115	A mild	V:1	definite	A. P	1944	Haematoma → gastro-intest. bleed.	10/59	15	80	12	26	
11	A mild	V:2	definite	Å. P	1953	None	7/59	23	88	11	5	
116	B severe	III:7	probable	B. O	1927	None	3/58	122	33	9	23	
116	B severe	III:8	potential	B. E.	1940	None	6/59	130	92	10	6	
							6/59	—	120	8	4	
							6/60	70	82	8	—	
118	B severe	II:2	definite	G. N	1907	None	2/59	75	55	10	19	
118	B severe	III:2	potential	I. D	1928	None	6/58	150	104	10	8	
							6/58	—	99	9	44	

Table I Cont.

Family No.	Haemophilia Type	Coordinate No.	Carrier state	Case Initials	Year of birth	Bleeding Symptoms	Date of invest. Mth/Yr	AHF % of normal	B factor % of normal	Coagulation time min.	Prothrombin consumpt. %	Comments
77	A severe	III.5	definite	M. S.	1922	None	1/61	40	80	20	—	
81	A moderate	IV:1	definite	U B J	1931	None	2/58	16	88	9	31	
86	A severe	III·6	potential	R. E.	1915	None	2/59	63	—	7	—	
							5/59	78	94	6	40	
							4/60	58	—	8	28	
86	A severe	III 7	probable	E. S	1917	None	6/57	44	—	6	—	
							12/57	19	105	6	24	
86	A severe	II.8	probable	E. L.	1893	None	12/57	85	150	10	4	
							12/57	100	—	—	—	
86	A severe	IV 10	potential	L. S.	1950	None	6/57	39	—	5	—	
87	A moderate	III 7	probable	E. L.	1884	None	8/57	176	—	3	—	
							10/57	163	—	—	—	
87	A moderate	IV 1	potential	G. B.	1912	None	2/59	107	112	6	—	
87	A moderate	IV.2	probable	M. F	1916	None	6/57	31	—	5	—	
							8/57	25	—	6	20	
							10/57	37	—	—	—	
							2/58	43	—	5	—	
							5 58	26	86	8	8	
87	A moderate	V.3	potential	B. F	1945	None	6/57	107	—	4	—	
87	A moderate	V 4	potential	K. F	1948	None	6/57	173	—	6	—	
88	A severe	III:2	probable	A. K.	1913	None	12 57	57	130	8	4	
							5 58	23	124	6	7	
89	A severe	III.3	probable	V. A.	1912	Haematoma	10 57	18	—	7	13	
							12 57	13	110	8	3	
							4 61	22	—	10	—	
89	A severe	IV.3	potential	V. L.	1937	Haematoma + epistaxis	12 57	25	75	7	6	
							5 58	23	—	7	6	
90	A severe	III 11	definite	H. B.	1930	Metrorrh. + postop. bleed.	11 57	61	—	7	8	
							1 58	45	94	—	—	
							5 58	—	—	5	20	
93	A mild	IV:2	potential	I. G.	1932	None	4 60	126	71	10	5	
95	A severe	III 1	definite	A. M.	1918	None	5 57	55	100	9	7	

Table I Cont.

Family			Carrier state	Case		Bleeding Symptoms	Date of invest. Mth/Yr	AHF % of normal	B factor % of normal	Coagulation time min.	Prothrombin consumpt. %	Comments
No	Haemophilia Type	Coordinate No.		Initials	Year of birth							
96	A mild	II:10	probable	E. A.	1906	None	3/57	86	—	7	12	
99	B severe	II:6	definite	K. G.	1914	None	3/60	70	43	10	28	
104	A mild	III.7	definite	H. V	1902	None	11/58	34	98	5	—	
104	A mild	IV 4	potential	S. S.	1930	None	1/59	118	85	8	17	
							1/59	105	—	8	—	
105	A severe	III:3	definite	R. B.	1928	None	1/58	42	66	11	28	
106	A severe	II:4	probable	S. J	1908	None	8/57	23	—	8	8	
108	A severe	II 6	probable	E. V	1912	None	11 57	46	100	5	0	
110	B severe	IV 9	definite	E. H.	1915	None	7 57	55	30	9	6	
							7/57	58	—	11	—	
							5 58	61	55	11	6	
							6 60	90	33	13	—	
							7 60	53	40	—	—	
112	B severe	III.6	definite	E. J	1903	None	11/57	83	52	10	11	
							6 58	84	59	11	21	
112	B severe	III.5	potential	E. F	1898	None	6 58	80	76	12	2	
112	B severe	IV:2	potential	H. F	1941	None	6 58	160	123	10	10	
114	A severe	II.2	potential	N. L.	1929	Tooth extract.	5 60	41	—	9	24	
							5 60	34	—	8	—	
114	A severe	II:6	definite	E. J.	1934	None	9 59	100	75	17	6	
							9 59	81	—	21	—	
							5 60	61	—	8	21	
							5 60	75	—	—	28	
							5 60	44	—	—	—	
115	A mild	V 1	definite	A. P	1944	Haematoma — gastro-intest bleed	10 59	15	80	12	26	
115	A mild	V 2	definite	A. P	1953	None	7 59	23	88	11	5	
116	B severe	III:7	probable	B O	1927	None	3 58	122	33	9	23	
116	B severe	III.8	potential	B. E.	1940	None	6 59	130	92	10	6	
							6 59	—	120	8	4	
							6 60	70	82	8	—	
118	B severe	II 2	definite	G N	1907	None	2 59	75	53	10	19	
118	B severe	III:2	potential	I D	1928	None	6 58	130	104	10	8	
							6 58	—	99	9	44	

Table I Cont.

Family No.	Haemophilia Type	Coordinate No.	Carrier state	Case Initials	Year of birth	Bleeding Symptoms	Date of invest. Mth/Yr	AHF % of normal	B factor of normal	Coagulation time min.	Prothrombin consumpt. %	Comment
118	B severe	III 3	definite	A.G.P	1930	Non	4/58 6/58	75 100	58 49	6 8	43 60	
118	B severe	III-6	definit	M. P	1937	None	2/59 2/59	70 —	60 63	7 7	26 —	
118	B severe	IV 1	potential	E. D	1951	None	6/58	57	54	8	12	
118	B severe	III 4	potential	S. G	1931	None	2/59 3/59	96 —	56 45	12 —	18 —	
120	A severe	IV 2	definite	A. J	1924	None	6/58	36	56	14	63	
123	A mild	III.23	definite	I. S.	1927	None	7/58 8 58	47 51	83 —	7 9	59 25	
126	A mild	III 3	definite	K. J	1925	Haematoma + bleed after partus	10 58	15	110	15	25	
127	A mild	III.2	defin t	S. F	1914	None	5 59	38	83	5	28	
133	A mild	IV 16	defin t	A. B	1943	None	1 61	31	85	11	17	
137	A mild	IV 2	potential	I. K.	1932	None	2 59 5 59 3 60	86 95 85	140 — —	— 10 8	35 22 —	
139	B mild	II 12	probabl	S. F	1902	Haematoma + bleed. after tooth extract.	3 60	150	30	9	92	
139	B mild	III 15	probable	E. E.	1924	Haematoma + bleed. after tooth extract	3 60	72	20	11	26	
139	B mild	IV 2	potential	S. E.	1944	Haematoma + epistaxis + gastro-intest bleed.	3 60	125	23	11	22	
139	B mild	IV 3	potential	M. E.	1944	None	3 60	65	100	9	39	

Table I Cont.

Family			Carrier state	Case		Bleeding Symptoms	Date of invest. Mth/Yr	AHF % of normal	B factor % of normal	Coagulation time min.	Prothrombin consumpt.	Comments
No.	Haemophilia Type	Coordinate No.		Initials	Year of birth							
139	B mild	IV:5	potential	L. E.	1954	None	3/60	75	88	13	50	
140	A severe	III:6	probable	B. B.	1926	None	11/57	46	156	—	—	
							12/57	48	—	6	—	
141	A mild	III:1	potential	B. O.	1916	None	5/59	60	148	9	14	
							6/59	90	100	7	—	
							12/59	95	—	—	—	
							4/60	140	93	6	11	
141	A mild	III:4	potential	S. F.	1912	None	6/59	172	99	7	—	
141	A mild	IV:3	definite	M. F.	1944	Haematoma	5/59	21	116	14	7	
141	A mild	IV:5	definite	S. F.	1930	Haematoma + epistaxis	5 59	38	106	9	—	
147	A severe	III:10	potential	V. S.	1931	Haematoma	11/59	20	93	13	13	
							12 59	26	—	13	—	
147	A severe	III:11	potential	E. L.	1935	None	1 61	80	62	10	20	
							1 61	90	—	—	—	
150	B moderate	IV:1	definite	S. J.	1929	None	3 60	37	15	7	15	
							5/60	43	23	9	25	
150	B moderate	IV:2	definite	H. E.	1934	Tooth extract.	2 60	44	13	9	128	
							3 60	58	17	9	12	
150	B moderate	IV:4	definite	E. C.	1943	None	3 60	51	22	11	23	
167	B mild	III:2	definite	M. Ö.	1941	None	11 60	135	59	11	28	
167	B mild	III:1	definite	K. C.	1937	None	11 60	260	71	14	53	gra- [illegible] IV
173	B moderate	III:7	probable	G. B.	1930	Haematoma	3 60	88	20	15	0	
							3 60	—	25			
							8 60	—	49			
							1 61	—	53			
173	B moderate	IV:3	potential	Y. B.	1931	None	3 60	85	20	11	0	
							3 60	—	28	—	—	
176	B severe	III:12	probable	M. L.	1931	None	8 60	71	32	8	19	

Altogether 32 potential carriers were investigated. Normal AHF values were recorded in 16 of these females. Low AHF values, ranging from 20 t 60 per cent of normal, were recorded in the remaining 16.

Table I Cont

Family			Carrier state	Case		Bleeding Symptoms	Date of invest. Mth/Yr	AHF % of normal	B factor % of normal	Coagulation time min.	Prothrombin consumpt. %	Comment
No.	Haemophilia Type	Coordinate No.		Initials	Year of birth							
118	B severe	III 3	definite	A.G.P.	1930	None	4/58 6/58	75 100	58 49	6 8	43 60	
118	B severe	III 6	definite	M. P.	1937	None	2/59 2/59	70 —	60 63	7 7	26 —	
118	B severe	IV 1	potential	E. D.	1951	None	6/58	57	54	8	12	
118	B sever	III 4	potential	S. G.	1931	None	2/59 3/59	96 —	36 45	12 —	18 —	
120	A severe	IV 2	definite	A. J.	1924	None	6/58	36	56	14	63	
123	A mild	III 23	definite	I. S.	1927	None	7/58 8/58	47 51	83 —	7 9	59 25	
126	A mild	III 3	definite	K. J.	1925	Haematoma + bleed after partus	10/58	15	110	15	25	
127	A mild	III 2	definite	S. F.	1914	None	5/59	38	83	5	28	
133	A mild	IV 16	definite	A. B.	1943	None	1/61	31	83	11	17	
137	A mild	IV 2	potential	I. K.	1932	None	2/59 5/59 3/60	86 93 85	140 — —	— 10 8	53 22 —	
139	B mild	II 12	probable	S. F.	1902	Haematoma + bleed. after tooth extract.	3/60	150	30	9	92	
139	B mild	III 15	probable	E. E.	1924	Haematoma + bleed. after tooth extract.	3/60	72	20	11	26	
139	B mild	IV 2	potential	S. E.	1944	Haematoma + epistaxis + gastro-intest. bleed.	3/60	125	23	11	22	
139	B mild	IV 3	potential	M. E.	1944	None	3/60	65	100	9	39	

Table I, Cont.

Family			Carrier state	Case		Bleeding Symptoms	Date of latest Mth/Yr	AHF % of normal	B factor % of normal	Coagulation time min.	Prothrombin consumpt.	Comments
No.	Haemophilia Type	Coordinate No.		Initials	Year of birth							
139	B mild	IV:5	potential	L. E.	1934	None	3/60	73	88	13	50	
140	A severe	III:6	probable	B. B.	1926	None	11/57	46	156	—	—	
							12/57	48	—	6	—	
141	A mild	III:1	potential	B. O	1916	None	5/59	60	148	9	14	
							6 59	90	100	7	—	
							12/59	93	—	—	—	
							4 60	140	93	6	11	
141	A mild	III:4	potential	S. F	1912	None	6/59	172	99	7	—	
141	A mild	IV:3	definite	M. F	1944	Haematoma	5 59	21	116	14	7	
141	A mild	IV:5	definite	S. F	1950	Haematoma + epistaxis	5 59	38	106	9	—	
147	A severe	III.10	potential	V. S.	1931	Haematoma	11/59	20	93	13	13	
							12 59	26	—	13	—	
147	A severe	III.11	potential	E. L.	1935	None	1 61	80	62	10	20	
							1 61	90	—	—	—	
150	B moderate	IV 1	definite	S. J	1929	None	3 60	57	15	7	15	
							5 60	45	25	9	25	
150	B moderate	IV 2	definite	H. E.	1934	Tooth extract.	2 60	44	13	9	128	
							3 60	58	17	9	12	
150	B moderate	IV 4	definite	E. C.	1943	None	3 60	51	22	11	23	
167	B mild	III.2	definite	M. O.	1941	None	11 60	135	39	11	28	
167	B mild	III 1	definite	K. C.	1937	None	11/60	260	71	14	53	grav mens IV
173	B moderate	III 7	probable	G. B	1930	Haematoma	3 60	88	20	15	0	
							3 60	—	25			
							8 60	—	49			
							1 61	—	33			
173	B moderate	IV 3	potential	Y. B.	1951	None	3 60	83	20	11	0	
							3 60	—	28	—	—	
176	B severe	III.12	probable	M. L.	1931	None	8 60	71	32	8	19	

Altogether 32 potential carriers were investigated. Normal AHF values were recorded in 16 of these females. Low AHF values, ranging from 20 to 60 per cent of normal, were recorded in the remaining 16.

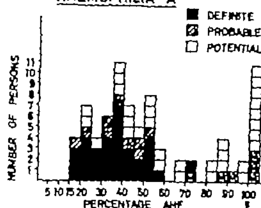


Fig 1

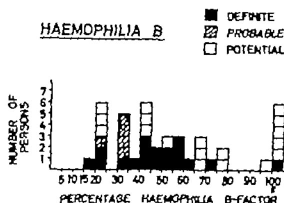


Fig 2

Of the 56 carriers showing low AHF values 7 had a slight prolongation of the coagulation time, and 9 had a pathological prothrombin consumption test. The level of haemophilia B factor in the investigated carriers of haemophilia A ranged from 56 to 160 per cent of normal The values found for other coagulation factors were within normal ranges.

Carriers of haemophilia B Altogether 15 of the 18 definite carriers of haemophilia B showed low B factor values ranging from 15 to 60 per cent of normal (tables I and II fig 2) Normal B factor values were recorded in the 3 remaining definite carriers *i e.* case III 8 in family 56 case III 6 in family 118 and case III 1 in family 167 The first woman A. J born in 1900 investigated on four

Table II

Type of carrier	Females belonging to haemophilia A families		Females belonging to haemophilia B families	
	No. of cases	Low AHG	No. of cases	Low B factor
Fertile age				
Definite	28	27	15	13
Probable	7	7	4	4
Potential	24	12	11	5
Postmenopausal				
Definite	4	3	3	2
Probable	7	2	1	1
Potential	3	2	4	0
Before menarche				
Definite	1	1	0	
Potential	5	2	3	2

different occasions, had values between 90 and 133 per cent. In the second, M P born in 1937 the values were between 60 and 65 per cent. The last K. C. born in 1937 had a B factor value of 71 per cent she was in the 5th month of pregnancy All 5 probable carriers had low B factor values.

In 7 of the 18 potential carriers, the value for the B factor was low ranging from 20 to 55 per cent

In 15 of the cases investigated, the haemophilia B factor was determined on repeated occasions The women who were low in B factor had consistently low values.

Of the 27 carriers with low B factor values, 9 had a slightly prolonged coagulation time and 4 pathological prothrombin consumption The AHF value of the investigated carriers of haemophilia B ranged from 68 to 160 per cent except in six cases (IV 9 in family 110

IV 1 in family 118 IV 1 IV 2 and IV 4 in family 150 III 1 in family 167) The three definite carriers belonging to family 150 showed AHF values ranging from 37 to 58 per cent. It must be pointed out that the male members of this family had in addition to their low B factor content (4 per cent of normal) AHF values of 40—50 per cent of normal. Case III 1 family 167 had an AHF value as high as 260 per cent. She was pregnant.

Discussion

As pointed out in our previous paper (12) a number of reports have appeared on the possibility of proving the heterozygous state in female carriers of haemophilia by investigating their plasma content of AHF and B factor by various methods. Several authors hold the view that a low AHF content cannot be demonstrated in carriers of haemophilia A (cf. 12) Others have found low AHF content in some carriers, but have been unable to demonstrate low AHF values consistently (cf. 12 see also 3 5 13 14)

Our investigation of 28 definite and probable *carriers of haemophilia A* showed the possibility of detecting female carriers of fertile age provided that a eligible method is used for the assay of AHF in plasma. We used method based on the ability of the plasma in dilution to correct the recalcification time of haemophilia A plasma. W found that it was important to assay the plasma in sufficiently high dilution. Careful collection of the blood samples, as well as the use of a standard plasma collected simultaneously and handled in exactly the same way as the plasma to be tested, proved to be of the utmost importance for obtaining reliable results with this method.

If our previous investigation is included, the plasma AHF content has hitherto been studied in 33 definite and 14 probable carriers of haemophilia A, as well as in 32 females without haemophilic sons, but with a 50 to 25 per cent genetic chance of being carriers. The object of this extended study was to ascertain the possibility of determining in the individual case, if overlapping of normal individuals with AHF at the lower level of the normal range and of carriers with only inappreciably decreased AHF values does, in fact, constitute a difficulty in evaluating whether or not the individual is a carrier The AHF was assayed only by the recalcification method on haemophilia A plasma. With this method individuals having an AHF plasma content of < 60 per cent can be regarded as low in AHF

The AHF was found to be low in 40 of our 47 definite and probable carriers of haemophilia A, ranging from 15 to 60 per cent of normal. Of the 7 carriers with normal AHF values, 6 were over the menopause. The only definite carrier of fertile age showing a normal AHF value was case II 6 in family 114 Her values varied between 44 and 100 per cent. This woman appeared to be in good health, and was not pregnant. One of her sisters without haemophilic sons was, on the other hand, low in AHF The mean AHF value for definite and probable carriers of fertile age (35 cases) was 37 per cent. This value is significantly lower than the mean value in the normal group.

As pointed out, the mean plasma AHF content in a group of normal healthy women over the menopause was found to be significantly higher than in a group of normal women of fertile age (cf Methods) This might explain why we were

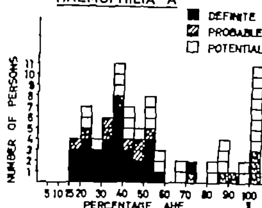


Fig 1

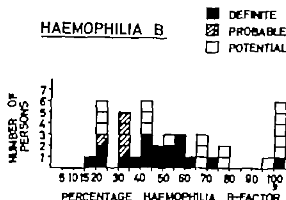


Fig 2

Of the 56 carriers showing low AHF values 7 had a slight prolongation of the coagulation time, and 9 had a pathological prothrombin consumption test The level of haemophilia B factor in the investigated carriers of haemophilia A ranged from 56 to 160 per cent of normal. The values found for other coagulation factors were within normal ranges.

Carriers of haemophilia B Altogether 15 of the 18 definite carriers of haemophilia B showed low B factor values ranging from 15 to 60 per cent of normal (tables I and II fig 2) Normal B factor values were recorded in the 3 remaining definite carriers, *i e* case III 8 in family 56 case III 6 in family 118 and case III 1 in family 167 The first woman A. J born in 1900 investigated on four

Table II

Type of carrier	Females belonging to haemophilia A families		Females belonging to haemophilia B families	
	No. of cases	Low AHG	No. of cases	Low B factor
Fertile age				
Definite	28	27	15	13
Probable	7	7	4	4
Potential	24	12	11	3
Postmenopausal				
Definite	4	3	3	2
Probable	7	2	1	1
Potential	3	2	4	0
Before menarche				
Definite	1	1	0	
Potential	5	2	3	2

different occasions, had values between 90 and 133 per cent. In the second, M P born in 1937 the values were between 60 and 63 per cent The last, K. C. born in 1937 had a B factor value of 71 per cent she was in the 5th month of pregnancy All 5 probable carriers had low B factor values.

In 7 of the 18 potential carriers, the value for the B factor was low ranging from 20 to 55 per cent

In 15 of the cases investigated the haemophilia B factor was determined on repeated occasions. The women who were low in B factor had consistently low values.

Of the 27 carriers with low B factor values, 9 had a slightly prolonged coagulation time and 4 pathological prothrombin consumption The AHF value of the investigated carriers of haemophilia B ranged from 68 to 160 per cent, except in six cases (IV 9 in family 110

carriers over the menopause had low B factor values. It was apparent from the methods that a group of healthy women of corresponding age did not show higher B factor values than the normal group as was the case for AHF. No explanation can be given of the normal value for the B factor recorded in the woman in question.

In several of the carriers, the B factor was determined on repeated occasions. The values obtained were consistently low. It is known that the value of the B factor can fall in connexion with liver disease, but no such disease was known in the tested subjects.

The B factor content of the plasma was also investigated in 18 potential carriers of haemophilia B having no haemophilic son. Seven of these were low in B factor and might be considered as carriers. The distribution of normal and low B factor values in this series is in good agreement with that expected from the genetic point of view.

With the recalcification method we used for assay of the B factor it seems possible to state that demonstration of a low value in a woman belonging to a family with haemophilia B does, in fact, mean that she is a carrier. If normal values are obtained, it is most likely that the woman is normal, although the possibility that she is a carrier cannot be completely ruled out.

Summary

An enlarged study of carriers of haemophilia A and B has been made in connexion with an investigation of 180 Swedish haemophilic families.

Altogether 33 definite carriers of haemophilia A, 14 probable (with one haemophilic son but no family history) and 32 potential carriers were examined. Significantly low values for the antihaemophilic factor (AHF or factor VIII) were found in 34 of 35 definite or probable carriers of fertile age, and in 16 of 32 potential carriers.

A study was also made of 18 definite, 5 probable and 18 potential carriers belonging to haemophilia B families. Low haemophilia B factor (factor IX) values were recorded in 20 of 23 definite or probable carriers, and in 7 of 18 potential carriers.

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unable to demonstrate low AHF values in all the carriers over the menopause.

The AHF content was investigated in females who might possibly be carriers of haemophilia A, i e potential carriers (fig 1)

From the genetic point of view this series of female relatives of haemophiliacs can be expected to contain about 50 per cent normal individuals and 50 per cent carriers The AHF content was found to be significantly low in 16 of the 32 potential carriers investigated

Taken collectively the results of our investigation of carriers of haemophilia A have provided evidence that such carriers of fertile age can be traced by demonstration of low AHF values. When potential carriers are investigated, they must be tested on repeated occasions. In our opinion consistently low AHF values are a strong argument for the woman in question being a carrier If on the contrary normal values are obtained on repeated occasions, we believe that the woman in question is, in all probability not a carrier It should however be recalled that we found one woman among the 35 definite and probable carriers of fertile age who had a low AHF value on one occasion only

In addition to the review given by Nilsson et al. 1959 (12) new investigations have recently appeared. Bentley & Krivit (3) studied the plasma AHF level in 21 definite and 5 probable carriers of haemophilia A by a modification of the erythrocytin method of Quick They found abnormally low AHF values in 23 of the subjects.

In 1960 Rapaport et al (14) who used Pool Robinson's modification of the thromboplastin generation technique, stated that this assay could trace four out of five carriers in a potential carrier population Pitney & Arnold (13) using a thromboplastin generation assay found a lower AHF value in the carrier group than in the normal. The age of the carriers with normal AHF values was not stated in these reports.

Certain authors, using various methods, have succeeded in demonstrating a low B factor content in carriers of haemophilia B, although only one or two cases were investigated by each author (1 7 8, 15, 16) Didisheim, Ferguson & Lewis (6) recorded a low content of factor B in 4 of 14 definite carriers, and in 3 of 5 possible carriers. These authors used a thromboplastin generation assay Hiser et al. (10) could not demonstrate any decrease in the content of haemophilia B factor in their carriers by a method based on the ability of serum to correct haemophilia B plasma. Barrow Bullock & Graham (2) investigated 13 mothers with haemophilia B sons, using a recalcification method with cephalin They found low B factor values in 10 of these carriers.

In the present investigation of carriers of haemophilia B, we used a recalcification method on haemophilia B plasma, similar to that we used for the assay of AHF Of 23 definite and probable carriers of haemophilia B we found 20 to be low in B factor the values ranging from 15 to 60 per cent Of the three carriers with normal B factor values, one (III 6 family 118) had a borderline value of 60 and 63 per cent and one was pregnant This woman also had an AHF level as high as 260 per cent. In the third carrier (III 8, family 56 B factor values of 90 to 133 per cent of normal were recorded This 57 year-old woman had 3 sons with severe haemophilia B. No other cases of haemophilia were known in the family Three other

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Haemophilia in Sweden

III. Symptomatology with Special Reference to Differences between Haemophilia A and B

By

Olof Ramgren

The symptomatology of haemophilia was described by Grandidier (11) as early as 1877 and numerous studies on the subject have been published since then (1—10, 12—17, 19—30). Most of them have concerned the general bleeding manifestations, but few have compared the symptoms and signs in relation to the type of haemophilia and the level of the coagulation factors (13, 14, 21, 22).

The coagulation status of 176 Swedish haemophiliacs was studied in a previous paper (18). A classification was made into haemophilia A and B of severe, moderate and mild form, according to the patient's plasma level of antihaemophilic factor (AHF or factor VIII) or haemophilia B factor (factor IX):

Severe haemophilia A and B: AHF or B factor < 1 per cent of normal.

Moderate haemophilia A and B: AHF or B factor 1—4 per cent of normal.

Mild haemophilia A and B: AHF or B factor 5—25 per cent of normal.

The bleeding manifestations in these patients have now been studied, and the results are presented in tables I—IX. The clinical picture was compiled in every case from hospital records, personal examination and a questionnaire answered by the patient.

Results

I. First symptom of haemophilia

The age at onset of the first clinical symptoms of haemophilia according to the type of bleeding manifestation is presented in tables I and II. The dominating first symptom in both severe

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Table III Haemarthrosis in haemophilia A and B

Haemophilia Type	No. of cases	No. of cases with haemarthrosis	No. of cases with repeated haemarthrosis in						No. of cases with impaired joint function after haemarthrosis in					
			Ankle joints	Knee joints	Hip joints	Shoulder joints	Elbow joints	Wrist joints	Ankle joints	Knee joints	Hip joints	Shoulder joints	Elbow joints	Wrist joints
A severe	71	68	63	63	38	44	64	53	18	44	1	1	28	—
A moderate	28	23	22	23	13	10	19	14	2	6	—	—	2	1
A mild	34	16	8	11	6	3	6	5	—	—	—	—	—	—
B severe	19	19	17	18	10	11	17	13	6	11	—	2	6	1
B moderate	10	9	5	9	5	2	6	4	—	4	—	—	3	—
B mild	14	4	2	3	—	1	—	—	—	—	—	—	—	—

Table IV Bleeding manifestations other than haemarthrosis in haemophilia A and B

Haemophilia Type	No. of cases	Gastrointestinal haemorrhage		Renal haemorrhage		Cerebral haemorrhage	Dental haemorrhage	Post-operative haemorrhage	Wound haemorrhage
		Isolated	Repeated	Isolated	Repeated				
A severe	71	7	18	9	34	2	12	4	24
A moderate	28	3	4	10	9	—	17	8	15
A mild	34	4	8	3	8	—	23	12	10
B severe	19	4	6	4	8	—	10	—	5
B moderate	10	3	2	3	2	—	5	2	7
B mild	14	1	1	1	3	—	8	5	1

Comments

It is seen from tables I–IV that in both haemophilia A and B a correlation is present between the level of AHF and B factor respectively and the severity and incidence of bleeding manifestations. No difference could be demonstrated between the two types of haemophilia in this respect. A distinction can easily be made between severe and mild haemophilia on the basis of the clinical features. It may on the contrary be difficult to distinguish clinically the individual case of moderate haemophilia of either type from severe or mild haemophilia.

The general clinical picture is in agreement with the results of Sköld (26) in his investigation of Swedish haemophiliacs in 1944 and also with other publications concerning Scandinavian haemophiliacs (1 14 25 30). It has been stated by some authors (5 6 21) that haemophilia of type B is a clinically milder form than haemophilia A. The present investigation has shown that no difference existed between the severity of haemophilia A and B in the Swedish haemophiliacs in question. The clinical features were in fact, identical.

In *severe* haemophilia of both types, the dominating symptom was haemorrhage into the joints, which occurred repeatedly and caused impaired function with advancing age. As seen from table V there was generally only a moderate degree of impairment before 10 years of age, whereas joint function was always severely impaired after the age of 20, and produced disablement in

Table I Age at onset of first clinical symptoms in haemophilia A and B

Haemophilia Type	No. of cases	Years of age						
		Before 1	1—2	3—5	6—7	8—10	11—15	After 15
A severe	71	25	42	3	1	—	—	—
A moderate	28	5	9	4	5	2	2	1
A mild	34	1	10	9	2	1	2	9
B severe	19	7	12	—	—	—	—	—
B moderate	10	2	2	3	3	—	—	—
B mild	14	1	3	2	1	1	1	3

Clinical symptoms have not yet appeared in 2 cases (P F and N F family 64).

Table II First clinical symptom in haemophilia A and B

Haemophilia Type	No. of cases	Subcutaneous haemorrhage	Haemarthrosis	Gastro-intestinal haemorrhage	Renal haemorrhage	Dental haemorrhage	Wound haemorrhage	Post-operative haemorrhage	Nasal haemorrhage
A severe	71	36	18	2	—	2	11	1	1
A moderate	28	9	5	—	—	3	9	1	1
A mild	34	5	3	—	1	8	13		
B severe	19	12	4	—	—	—	3	—	—
B moderate	10	4	2	—	—	2	2	—	—
B mild	14	3	—	1	—	2	4	—	2

haemophilia A and B was subcutaneous haemorrhages, and the age at onset was generally before three years. In mild haemophilia A and B the dominating initial symptoms were dental and/or wound haemorrhages, and the age at onset was between 1 and 5 years Moderate haemophilia A and B generally had its onset before 8 years of age the first symptoms being subcutaneous and wound haemorrhages Thus, as far as the age at onset and initial clinical symptoms were concerned no difference was found between haemophilia A and B with a corresponding level of AHF or B factor

II Haemarthrosis

The findings of repeated haemarthrosis and impaired joint function in haemophilia A and B are recorded in table III

In both moderate and severe haemophilia A and B practically all the patients had a history of repeated haemarthrosis. In mild haemophilia A and B this applied to only half of the patients The proportion of repeated haemarthrosis and impaired joint function was the same in both haemophilia A and B of the corresponding degree of deficiency

III Bleeding manifestations other than haemarthrosis

As shown in table IV renal haemorrhage was the most common bleeding manifestation — apart from haemarthrosis — in both severe and moderate haemophilia A and B

In mild haemophilia of both types dental and/or postoperative haemorrhage was the most usual bleeding symptom

In the three forms of haemophilia classified according to the level of the coagulation factor in question, the clinical features can be summarized as follows, on the basis of the symptomatology

Severe haemophilia (AHF or B factor < 1 *per cent of normal*) The symptoms generally appear during the first two years of life, and the diagnosis is usually obvious at the first visit to hospital. Repeated haemarthroses occur in ankle, knee elbow and wrist joints at an early age, and result in impaired joint function after the age of 10. Moreover severe changes in the joints often give rise to moderate or severe disablement. Large subcutaneous, intramuscular and retroperitoneal haematomas appear spontaneously or after slight trauma. Episodes of haemorrhage from the renal and/or gastrointestinal tract are common. They require frequent hospitalization and often regular transfusions of blood or plasma. The coagulation time is appreciably prolonged, *i. e.*, to more than 30 minutes, whereas the bleeding time is normal.

Moderate haemophilia (AHF or B factor 1—4 per cent of normal) The first symptoms generally appear before 8 years of age. Haemarthroses are less frequent than in severe haemophilia and, as a rule, are restricted to a few joints, usually the knee or elbow. They do not lead to impaired function of the joint before middle age. Spontaneous haematomas in the subcutis, muscles and retroperitoneum are rare. Sporadic episodes of renal or gastrointestinal haemorrhage occur but require hospitalization only when the bleeding is profuse. Blood or plasma transfusions are usually required in the presence of haemarthrosis, and renal or gastrointestinal haemorrhage. The coagulation time is prolonged, ranging from 10 to 45 minutes.

Mild haemophilia (AHF or B factor 5—25 per cent of normal) In half of the cases the initial symptoms appear before 8 years of age, and in one-fourth of them in adolescence. Haemarthrosis occurs in not more than half of the cases, and only after moderate or severe trauma. It is restricted to one or two joints, and does not lead to impaired joint function. The bleeding episodes take place after tooth extraction or in connexion with minor and major surgery or only as repeated renal or gastrointestinal haemorrhage. Hospitalization is rare, and blood transfusions are needed only when bleeding is profuse. The coagulation time may be prolonged, but is within the normal range in half of the cases.

Summary

A study has been made of the bleeding manifestations in 176 patients with haemophilia A or B classified as severe, moderate or mild according to the plasma content of antihaemophilic factor (AHF or factor VIII) or haemophilia B factor (factor IX)

It is found that, as far as the severity of the bleeding manifestations is concerned, no difference is present between haemophilia A and B with a corresponding level of the coagulation factors in question

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Table V Haemarthrosis. Impaired joint function related to age in severe and moderate haemophilia A and B

	Year of birth	Age years	Severe haemophilia							Moderate haemophilia						
			No. of cases	Impaired function of joints						No. of cases	Impaired function of joints					
				Ankle	Knee	Hip	Shoulder	Elbow	Wrist		Ankle	Knee	Hip	Shoulder	Elbow	Wrist
Haemophilia A	1950—1959	1–10	22	4	5	—	—	—	—	3	—	—	—	—	—	—
	1940—1949	11–20	20	8	16	1	—	12	—	10	1	2	—	—	—	1
	1920—1939	21–40	20	4	17	—	—	11	—	8	—	3	—	—	1	—
	Before 1920	< 40	9	2	6	—	1	5	—	7	1	1	—	—	1	—
			71	18	44	1	1	28	—	28	2	6	—	—	2	1
Haemophilia B	1950—1959	1–10	5	1	—	—	—	—	—	2	—	—	—	—	—	—
	1940—1949	11–20	7	1	4	—	1	1	1	2	—	1	—	—	—	—
	1920—1939	21–40	4	2	4	—	—	3	—	4	—	3	—	—	3	—
	Before 1920	< 40	3	2	3	—	1	2	—	2	—	—	—	—	—	—
			19	6	11	—	2	6	1	10	—	4	—	—	3	—
Total			90	24	55	1	3	34	1	38	2	10	—	—	5	1

some cases. Severe disablement may however be present even at a lower age (cases IV 17 and IV 18 family 72)

The next most prominent symptom in severe haemophilia was haematuria which occurred on at least one occasion in about two-thirds of the patients. In several cases haematuria appeared after other bleeding manifestations, or in combination with them

Postoperative haemorrhage or haemorrhage after tooth extraction was not reported as often in the severe as in the moderate and mild forms of haemophilia This can be explained by the fact that severe haemophilia is clearly recognized at an early age consequently operations and dental extractions are not performed without careful protection by blood therapy Neither major nor minor surgery is done except on vital indications

In *moderate* haemophilia the clinical features were the same as in severe haemophilia except that the incidence of bleeding manifestations was generally lower although bleeding was as profuse as in severe haemophilia. It must be emphasized that moderate haemophilia is an arbitrary classification, and that this group undoubtedly contains many borderline cases.

In *mild* haemophilia only half of the patients had a history of haemarthrosis. These bleedings were slight and did not cause impaired joint function Mild haemophilia was characterized by postoperative and/or dental haemorrhage and by repeated bleeding from a definite site, *e g* gastrointestinal or renal haemorrhage Subcutaneous and intramuscular haemorrhages occurred only after moderate or severe trauma in contrast to moderate or severe haemophilia in which spontaneous haemorrhages were most common

Conclusions

In the present series of 176 Swedish haemophiliacs, no difference could be found between the clinical features in haemophilia A and B

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Cytochemical Investigations and Serum Vitamin B_{12} Determinations in a Case of Erythroleukaemia

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Erythroleukaemia was first described by di Guglielmo in 1917 (16, 17). The disease is rare, but not so rare as erythraemic myelosis. It is characterised by a refractory anaemia, pyrexia, stomatitis, haemorrhagic diathesis, lowered resistance to infection, sometimes slight enlargement of liver and spleen and a pathological preponderance of erythroblastic and myeloblastic activity in the bone marrow — generally combined with a peripheral erythroblastosis and myeloblastosis and, not uncommonly leukopenia. The disease progresses rapidly and is comparable in this respect with acute leukaemia.

The distinction between erythroleukaemia and on the one hand acute myeloblastic leukaemia, and on the other erythraemic myelosis is difficult and sometimes arbitrary (11 23).

Confusion with myeloblastic leukaemia can occur as result of the peripheral orthochromatic erythroblastosis sometimes seen in the latter condition, simulating erythroleukaemia. In such cases the diagnosis of erythroleukaemia is supported primarily by the presence of pathological multinuclear giant erythroblasts in the bone marrow and also to a certain extent by an erythroblast/myeloblast ratio exceeding one (23 35 40).

The borderline between erythroleukaemia and erythraemic myelosis is often indistinct despite the absence of malignant myeloid activity in the latter. Some writers even consider that there is no certain difference between these two diseases, and that erythraemic myelosis develops from or develops into erythroleukaemia (5 11 23 27 40). Thus Dameshek has adopted the term di Guglielmo's syndrome for both these conditions (5 10 11).

Cytochemical studies of the blood cells, and serum vitamin B_{12} studies have been reported in only a few cases of erythroleukaemia. In the case described below alkaline phosphatase staining of neu-

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b

Fig. 2. a) Two orthochromatic erythroblasts, one of which has two nuclei, and one plasma cell. May-Grünewald-Giemsa technique. × 2,350. b) The same field as a) stained with the PAS method. Positive reaction in the erythroblasts. Negative reaction in the plasma cell. × 2,350.



Fig. 3. PAS-positive erythroblast with multiple nuclei. × 2,700.



Fig. 4. Phagocytosis of erythrocyte by myelocyte. May-Grunewald-Giemsa technique. × 2,800.

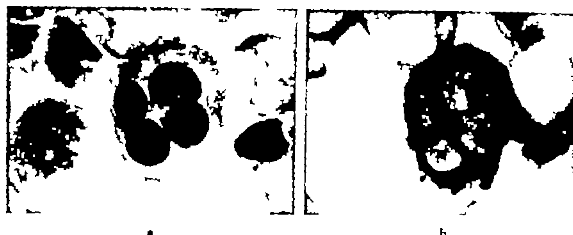


Fig. 1 a) Multiple nuclei in a basophilic erythroblast, surrounded by myeloblasts. May-Grünewald-Giemsa technique. × 1,240. b) The same field as a) stained with the PAS method. Intense positive reaction in the erythroblast. Negative reaction in the myeloblasts. × 1,240.

trophil leukocytes. PAS-staining of erythroblasts, neutrophil leukocytes and lymphocytes, iron staining of erythroblasts and serum vitamin B_{12} determinations were carried out. These investigations may be of diagnostic value in certain cases.

Case report

The patient was a 67 year-old moulder. His symptoms began on August 1st 1960 with increasing lassitude. Ten days later a focus of suppuration was discovered around the roots of two teeth, which were extracted. On August 13th he became pyrexic with a temperature of 39° C. He was given penicillin and sulphonamide preparation following which his temperature returned to normal after 5 days. Persistent anaemia led to his admission to the medical clinic, Lund on August 18th 1960.

Examination findings. Patient pale and tired looking. Mouth and throat — pale mucous membranes. Several badly decayed teeth. No lymphadenopathy. Abdomen — no palpable enlargement of liver or spleen. No evidence of haemorrhagic tendency. No other significant physical findings.

Plain X-ray of abdomen. Liver and spleen of normal size.

Chest X-ray. Normal.

ECG. Normal.

Laboratory findings on admission. Hb 6.3 g per cent, RBC 1.9 million per mm³. MCV 87 μ³, MCHC 38 per cent, haematocrit 14 per cent, platelets 237 000 per mm³, reticulocytes 0.8 per cent, WBC 1,900 per mm³. Diff. neutrophils 10 %, eosinophils 0 %, basophils 0 %, lymphocytes 57 %, monocytes 9 %, neutrophil myelocytes 2 %, myeloblasts 20 %, plasma cells 2 %, polychromatic normoblasts 2/100 WBC, erythrocytes showing punctate basophilia 9/100 WBC.

Sternal puncture. Early neutrophils 2 %, adult neutrophils 0 %, eosinophils 0 %, basophils 0 %, lymphocytes 11 %, monocytes 0 %, metamyelocytes 2 %, neutrophil myelocytes 6 %, eosinophil myelocytes 3 %, promyelocytes 21 %, myeloblasts 36 %, plasma cells 19 %, erythroblasts 256/100 WBC (pronormoblasts 3/100 WBC, basophil normoblasts 60/100 WBC, polychromatic normoblasts 189/100 WBC, oxyphil normoblasts 4/100 WBC). Numerous multinucleated giant erythroblasts (figs. 1—3). Erythrophagocytosis was seen (fig. 4).

Serum iron 166 μg %, serum bilirubin 0.3 mg %, Prothrombin index 88, Thymol turbidity 0.06 ext. Serum alkaline phosphatase 5 U, Fibrinogen 0.31 g %, Haptoglobin 185 mg %, Coombs test negative. Faecal urobilinogen 83.7 mg per day. Blood sugar determinations: values between 110 and 140 mg % — on one occasion a fasting value of 180 mg % was found and on two occasions the afternoon values were 190 and 200 respectively. A glucose tolerance test was not performed.

Electrophoresis. Marked reduction in albumin (2.83), marked alpha$_1$-increase (0.49)

b

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a b

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Sternal puncture Early neutrophils 2 %, adult neutrophils 0 %, eosinophils 0 basophils 0 lymphocytes 11 monocytes 0 %, metamyelocytes 2 neutrophil myelocytes 6 eosinophil myelocytes 3 %, promyelocytes 21 myeloblasts 36 %, plasma cells 19 % erythroblasts 256/100 WBC (pronormoblasts 3/100 WBC, basophil normoblasts 60/100 WBC, polychromatic normoblasts 189/100 WBC, oxyphil normoblasts 4/100 WBC) Numerous multinucleated giant erythroblasts (figs. 1—3) Erythrophagocytosis was seen (fig 4)

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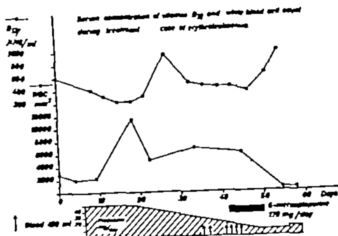


Fig. 5. Serum vitamin B_{12} normal or at the upper limit with slight terminal peak. No certain relation to WBC.

Special studies

Alkaline phosphatase staining was carried out by the method of Kaplow (29) (Merker & Hellmeyer modification (33)). The substrate was sodium-alpha-naphthyl-phosphate and the diazosalt "Variamin-blausalz (Hoechst). Neutrophil leukocytes were graded by the Merker & Hellmeyer score method (33). The score in the peripheral blood was 356 initially (normal 10—100). After steroid administration rise to over 400 (416—407—410) was observed. There was also marked staining in neutrophil leukocytes in the bone marrow.

PAS-staining (Periodic acid-Schiff reaction) was done by Hotchkiss method (38). Erythroblasts were scored as described by Hayhoe & Quaglino (24, 39) and neutrophil leukocytes and lymphocytes as described by Astaldi (2). In the bone marrow there was staining of numerous erythroblasts, proerythroblasts, b-normal giant erythroblasts and orthochromatic erythroblasts (figs. 1—3). As the disease progressed and after steroid therapy there was more intensive staining in the basophil erythroblasts and proerythroblasts (table I). I the peripheral blood too there were numerous stained erythroblasts.

Early in the disease the neutrophil leukocytes stained normally both in the bone marrow and in the peripheral blood. The stain intensity increased in the later stages of the disease to abnormal values (table II). This may be related to an abnormal glucose tolerance which unfortunately was never fully explored.

During the entire disease there was marked increase in PAS-positive granules in the lymphocytes (table II).

Iron staining was performed by Perl' method (38) (prussian blue). Orthochromatic erythroblasts contained relatively large amounts of iron granules. The occasional granule was seen in small number of proerythroblasts and basophil erythroblasts.

Serum vitamin B_{12} concentration. Serum vitamin B_{12} was estimated with Euglena gracilis as described by Killander (30) once or twice week during the entir disease (fig. 5). Normal values were obtained throughout. A certain degree of variation between the normal limits was seen during the course of the disease, but no correlation was noted between this and the other variables such as leukocytosis, fever variations in treatment etc.

Discussion

Cytochemical investigations have been done only in few cases of erythroleukaemia (5 11 14 23 24 39). Dameshek et al. (5 11) have in occasional cases noted positive PAS-staining in erythroblasts in di Guglielmo s syndrome, and in 1960 Quaglino & Hayhoe (24) described three cases of erythraemic myelosis and eight cases of erythroleukaemia with increased amounts of PAS-positive substance in the

Table I. PAS-pattern in erythroblasts in bone marrow graded from 0 to 3 with increasing staining intensity (24). Highly positive PAS-staining in both early and late erythroblasts

Date	Proerythroblasts and basophilic erythroblasts				
	0	1	2	3	Score
19.8	52	33	12	3	66
27.9	34	22	26	18	128
	Polychromatic and orthochromatic erythroblasts				
	0	1	2	3	Score
19.8	73	11	11	5	48
27.9	64	29	7	0	43

Table II. PAS-staining in neutrophils and lymphocytes in peripheral blood smears graded from 0 to 4 (2). Highly positive PAS-staining in lymphocytes and, late in the disease, in neutrophils

	Date	Neutrophil leukocytes	Lymphocytes
Patient	19.8	2.80	2.24
	26.9	2.87	1.96
	10.10	3.40	2.36
Average in smears from 18 normal persons, Mean ± S. E. M.		2.78 ± 0.08	0.87 ± 0.09

moderate alpha₂-increase (0.66) normal beta-fraction (0.77 %) marked diffuse gamma-increase (2.45 %). No characteristic myeloma band. Serum cholesterol 95 mg %. Serum vitamin B_{12} and cytochemical investigations, see below.

Course of disease

During his stay in hospital the patient had febrile periods. His tiredness increased. He developed widespread bruises and petechiae, and during the week before his death he developed large necrotic areas on the mucous membrane of his mouth, these being invaded by fungus mycelia.

He was treated with antibiotics (penicillin, terramycin, and chloromycetin, alternately), blood transfusions, steroids (Prednisone 30 mg progressively reduced to 15 mg daily) and for nine days towards the end of the illness with 6-Mercaptopurine (175 mg daily) all without favourable effect. Apart from temporary improvement after blood transfusions the anaemia advanced progressively. A mild leukocytosis of 10 700 was observed during a febrile period, but in the later stages of the disease there was a return to progressive leukopenia. The latter appeared some ten days after starting treatment with 6-Mercaptopurine. Progressive erythroblastosis was seen in the peripheral blood (max. 44 normoblasts/100 WBC of which 10 % were basophil and polychromatic erythroblasts, 25 oxyphil erythroblasts and 9 % erythroblast nuclei).

In sternal puncture material the number of multinuclear giant erythroblasts increased during the illness. In association with the terminal leukopenia there was decline in both erythropoiesis and myelopoiesis. The picture at this time was dominated by large abnormal myeloblasts. There was a moderate reticulocytosis during the whole course of the disease (about 3 per cent). Despite the use of steroids, thrombocytopenia progressed rapidly to 13,000. In the electrophoresis there was regression of the gamma-increase (1.39 %). Otherwise largely ISQ.

The patient died on October 10th 1960 after an illness of about 2½ months.

Post mortem. The findings were of a blastic leukaemia with leukaemic changes in the bone marrow, slight enlargement of liver and spleen and a suggestion of leukaemic changes in the lymph nodes. There was a solitary mandarinesized abscess in the right lobe of the liver — no evidence of bacterial or fungal infection. The lungs showed multiple patches of consolidation, and bilateral fibrinous pleurisy. There was cholesterolosis of the gall bladder.

Diagnosis. The course taken by the illness, together with the laboratory findings, indicate a diagnosis of erythroleukaemia with a terminal hypoplasia of the bone marrow dominated by myeloblasts.

to be augmented. Staining for iron in erythroblasts gave normal results. Serial B_{12} estimations were carried out, and variations within normal limits were seen during the course of the disease.

Cytochemical investigations can be of value in differentiating between erythroleukaemia and myeloblastic leukaemia with erythroblastosis.

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erythroblasts. Comparable increases occur only in thalassaemia and in severe iron deficiency anaemia. Lesser increases occur in haemolytic anaemia among other conditions (3 39) PAS-positive erythroblasts are uncommon in acute myeloblastic leukaemia (23) for which reason PAS-staining is of considerable interest in the differentiation of erythroleukaemia from myeloblastic leukaemia with erythroblastosis The similarity in PAS-pattern between erythroleukaemia and erythraemic myelosis lends support to Dameshek's use of the term di Guglielmo's syndrome

Increased PAS-intensity in neutrophil leukocytes and lymphocytes in erythroleukaemia has, as far as we are aware, not been previously described.

In neutrophil leukocytes such increases can also be seen in infections diabetes mellitus and polycythaemia vera (2 22 23) *In lymphocytes increased amounts of* PAS-stained granules have been observed in a variety of diseases, such as chronic lymphatic leukaemia, diabetes mellitus and infections of different sorts (4 31) The marked increase noted by us is comparable only with that found in chronic lymphatic leukaemia and in diabetic coma. The patient showed increased blood sugar levels but no signs of ketosis

PAS-positivity in erythroblasts neutrophil leukocytes and lymphocytes is probably due to glycogen or glycogen-like substances, since previous exposure to diastase markedly hinders or prevents staining (2 23 38) Whether staining intensity is a direct expression of the glycogen content of the cell, is, however uncertain (22)

Alkaline phosphatase staining of neutrophil leukocytes showed very high values, comparable only with those found in severe infections or lymphoblastic leukaemia (23 33)

Hayhoe has demonstrated values in erythroleukaemia varying from abnormally low to abnormally high In six such cases described by him there were high values in four and low values in two. In the latter two cases the diagnosis, for a variety of reasons, was considered doubtful Thus in established cases of erythroleukaemia only high values have hitherto been described Our case is in agreement with this. Since low values are seen in myeloblastic leukaemia, alkaline phosphatase staining can assist in differentiating between erythroleukaemia and myeloblastic leukaemia with erythroblastosis.

Iron granules are seen normally in erythroblasts and have also been noted previously in di Guglielmo's syndrome (1 5) The amount of granules found should agree closely with what is found normally at a given serum iron level (24 28) A rapid plasma clearance of injected Fe^{59} with a low iron incorporation in the erythrocytes has been found by Fisher et al. (15) They also noted an increased red cell destruction (Cr^{51} T/2-6 days)

Serum B_{12} has only been estimated previously in a few cases of erythraemic myelosis and erythroleukaemia (1 5 10, 11 15) Both high and low values have been observed Serial estimations in our case showed wide variations within normal limits, uncorrelated with other features of the disease (fig 5)

Summary

A case of erythroleukaemia is described PAS-staining intensity in erythroblasts, neutrophil leukocytes and lymphocytes was increased. Alkaline phosphatase activity of neutrophil leukocytes was shown

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Roentgenological Department, Central Military Hospital and Gastroenterological Research Laboratory Charles University Prague ČSSR

Contribution of Splenoportography to the Diagnosis of Diseases of the Pancreas

I Tumorous Diseases

By

Josef Rösch and Karel Herfort

The diagnosis of diseases of the pancreas by splenoportography is rendered possible by the relationship of the pancreas to the large veins of the portal system, mainly the lienal vein and the portal vein which are visualized in splenoportography. Both these veins are in the close vicinity of the pancreas. The lienal vein runs first along the upper edge of the tail of the pancreas, then turns to the posterior surface of its body and behind the cervix or head of the pancreas it passes to the portal vein. Sometimes there is a small groove in the pancreas accommodating the lienal vein, sometimes the vein passes directly through the parenchyma of the pancreas (Douglas). The portal vein in its initial portion lies close to the posterior and sometimes also to the upper edge of the head of the pancreas. A pathologically altered pancreas therefore causes very soon changes in these veins, their displacement, deformation, stenosis or complete occlusion, and splenoportography which detects these changes thus provides reliable and valuable information on the pancreas.

A number of authors have already drawn attention to the possibilities of splenoportography in the diagnosis of pancreatic tumours. The experience of different authors, however, disagrees in many respects and their views as regards the value of splenoportography in this connection also differ. Scholz, Cernini, Anacker and others consider splenoportography merely a supplementary method with a small diagnostic value because in their patients, most of whom suffered from tumours of the head of the pancreas, they frequently encountered a normal picture and changes were found only in extensive tumours. Leger, Leroux, Cacciari, Catalano, Franchini, Mosely as well as most other authors, however, very frequently found changes on the splenoportal

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Table I

Site and character of tumour	No. of pat.	No. of examin.	No. confirmed by operation or necropsy
Tumours of papilla of Vater and small operable tumours of head of pancreas	7	7	7
Inoperable tumours of head	13	13	13
Inoperable tumours of body	12	12	10
Inoperable tumour of tail	2	2	2
Postnecrotic pseudocysts of pancreas	10	16	10

trunk and they include splenoportography among the main diagnostic methods for tumours of the pancreas. They advocate splenoportography *not only for its* diagnostic value but also because it helps to determine the operability of the tumour. In the present paper we seek to define, from these reports and our own experience, the range of possibilities of splenoportography and to show its contribution in this difficult sphere of diagnostic work.

Examination technique

We have introduced our own method and the main part of the examination is carried out under skiascopic control. The examinations are made on an Elema seriograph.

In the course of the preparation we rule out the sensitivity of the patient to iodine, the blood coagulation and bleeding time is examined, if necessary ascites is drained and to icteric patients vitamin K injections are administered for 4—5 days. Before the examination we instruct the patient how to hold his breath in the maximum inspirium for 20 seconds because during the apnoic period we carry out the entire examination proper, i. e. the puncture of the spleen, the injection of the contrast material and the seriographic pictures. During the examination the patient lies on his back. First we determine skiascopically the size and site of the spleen and the site of puncture, which in the normally sized spleen is usually in the 9th or 10th intercostal space in the median to posterior axillary line. To improve the visualization of the spleen, sometimes insufflation of the large intestine with air is used. At the marked site the thoracic wall is anaesthetized and pierced with an injection needle 1.5 mm in diameter which is connected with the syringe by means of a short rubber tubing. After introducing the needle into the chest wall the patient takes a maximum deep breath, holds his breath and under visual skiascopic control the needle is introduced into the spleen into the region of the hilus. To determine the position of the needle 1—2 ml of contrast substance are injected on trial, and if the needle is properly in situ, this is immediately drained into the lienal vein. During the subsequent 3—4 seconds we add the total remaining amount of the contrast substance. Usually 20 ml of 70 diodin are used. During the examination 8—9 X-ray pictures are taken. The first four in one-second intervals, the next two in two-second intervals and the last two to three pictures in three-second intervals. After the examination the patient remains in bed for 12 hours.

Normal picture

The contrast substance injected into the spleen forms a small depot round the point of the needle which remains in the spleen for 15—20 minutes. The other contrast substance is drained immediately and fills the lienal vein which in front of the spine passes into the portal vein leading into the hilus of the liver. The course of the splenoportal trunk is regular, its filling is homogeneous or regularly grooved and its outline is smooth and sharply defined. Only at the orifice of the superior mesenteric vein there is sometimes a small defect which spreads to the portal vein and which is due to the inflow of blood not containing the contrast substance. From the portal vein the contrast substance is drained to the hepatic tributaries and the

extrahepatic system is rapidly emptied. Usually already 3-4 seconds after injection, the extrahepatic part of the circulation is emptied. Only where more contrast substance is left in the spleen, small amount sometimes remains in the lienal vein this is, however never homogeneous and forms usually only small stripes with slight contrast properties on the upper or lower wall of the vein. This residual filling of the lienal vein can be usually found only on the outer and central part of the ein, as later the contrast substance is diluted with blood and can no longer be visualized. From the portal hepatic branches the contrast substance is drained to the sinusoids and the intensity of the hepatic shadow is enhanced the stage of liver opacity develops. The opacity is homogeneous and diffuse

This normal picture was observed in 50 patients, who did not suffer from disease of the pancreas or spleen and where there were no substantial changes on the liver In thirty of these patients the normal finding on these organs was confirmed surgically and in two also on necropsy These patients were operated on for gastric or duodenal ulceration, polyp or carcinoma of the stomach without signs of metastases, for cardiospasm or cholelithiasis.



Fig. 1 Operable tumour of head of pancreas. Slight pressure changes on lienal vein before spine with reduced contrast filling Winding of lienal vein in its left portion due to displacement of spleen by puncture needle.



Fig 2. Status after pancreatoduodenectomy for carcinoma of head of pancreas made four years ago. Knee-shaped bend of lienal ein before its orifice into portal ein retrograde filling of gastric and retroper toneal eins.

Material and results

A total of 44 patients were examined, on whom 50 splenoportographic examinations were made. A more detailed classification of the patients according t the character and si of the tumour is given in table I Postnecrotic pseudocysts were listed with tumours because by their nature they are much closer to them than to inflammatory conditions.

Tumour of the papilla of Vater and small operable tumours of the head of the pancreas

I six patients the umour originated either directly in the papilla of Vater or in the parenchyma of the pancreas in it vicinity Most tumours were small, did not spread t the neighbouring organs and tissues and were associated with typical clinical symptoms, mainly obstructiv jaundice A radical removal of the tumour was possible in three patients where pancreatoduodenectomy was carried out. I further three patients only palliativ cholecystoduodenoanastomosis was made in iew of the advanced age and poor general condition of the patients which argued *against radical operation.*

The splenoportographic picture was only little altered. No changes were found in the extrahepatic system in two patients. In another tw patients there were pressure changes on the lienoportal trunk in front of the spine — in one of these patients the pressure was not convincing and only slight the lower edge of the trunk. I the second patient the lienoportal trunk in front of the spine was slightly compressed in downward and dorsal



Fig 3 Inoperable tumour of head of pancreas. Considerable narrowing of portal vein marked collateral circulation to the stomach large intestine and retroperitoneum



Fig 5 Inoperable tumour of head of pancreas. Complete occlusion of portal vein with signs of penetration of tumour into lienal vein and upper mesenteric vein. Filling of newly formed hepatopetal collateral veins in region of hepatoduodenal ligament small collateral veins also in gastric region.



Fig 4 Inoperable tumour of head of pancreas Advanced occlusion of right half of lienal vein and portal vein with signs of direct penetration of tumour into vein. Marked enlargement of lienal vein before occlusion, marked hepatopetal collateral circulation via gastric and large intestinal veins (oblique projection)

direction and very slightly also in the sides. In the remaining two patients where on operation changes suggesting pancreatitis were found, splenoportograph revealed changes suggesting an inflammatory enlargement of the pancreas. Apart from these changes in the extrahepatic system, in four patients changes in the liver were found which were due to secondary cholostasis. In the stage of venous filling the hepatic branching was poor the smaller and medium-sized branches had a rigid and stretched appearance. In the stage of opacity there appeared some striped defects, ovoid to circular but usually only small. The extent of these changes corresponded to the stage of cholostasis and the enlargement of the bile ducts.

An interesting splenoportographic picture was found in a patient where four years previously pancreatoduodenectomy was performed for a histologically confirmed tumour of the head of the pancreas. The lienal vein in this patient was enlarged along its entire course, only in front of the left half of the spine it was narrower knee-shaped and filled in a retrograde manner the collateral branches leading to the stomach and retroperitoneum. Since the patient has been well for more than two years after the examination and lacks signs of relapse of the tumour the deformation of the lienal vein and its partial obstruction were no doubt, due to postoperative adhesion of surrounding tissu

On oral examination of the stomach and duodenum by the commonly used method, in three patients a slight and not convincing enlargement of the duodenal fenestra was found, in one patient considerable pressure on the duodenum caused by the tumour and in another patient pressure caused by the enlarged biliary pathways. In one patient the picture of the stomach and duodenum was normal.

Inoperable tumours of the head of the pancreas

The cause of inoperability was usually the size of the tumour which frequently penetrated through most of the head of the pancreas and sometimes even into the surrounding tissues. The inoperability of the tumour was promoted in some patients by the general poor condition of the patient and metastases in the nodes and liver. The clinical manifestations of the tumour and the complaints of the patients were in most instances typical — mainly digestive disturbances, loss of weight, later also pains and frequently but not always, jaundice.

Splenoportography revealed very varied pictures with large scale of changes from normal picture to a complete occlusion of the veins. I two patients where the tumour grew from the anterior surface of the head of the pancreas nd spread mainly in a forward direction, the splenoportographic picture was quite normal and the lienoportal trunk was not at all impaired. Also the other examinations, in these tw patients, including those of the stomach and duodenum, did not show any substantial deviations and the tumour as detected in one patient on laparotomy in the second only necropsy In the remaining patients the splenoportograms showed mainly changes in the splenoportal trunk. I tw patients w found narrowing of the lienal vein in front of the spine with uneven edges caused by the direct penetration of the tumour In tw further patients where the tumour spread into the hepatic hilus, splenoportographs revealed partial occlusion of the portal ein with marked narrowing due to pressure I the remaining seven patients w evealed complete occlusion which in three patients was at the site of origin of the portal ein, in three patients on the lienal ein at the left edge of the spine in one patient where the tumour affected also the body of the pancreas, there was complet occlusion of the lienal vein lready at the hilus of the spleen I three patients splenoportography revealed in addition metastases in the liver

The splenoportographic picture of the occlusion was characterised by an amputation of the ein, the outlines of which were sometimes uneven and dentate as well as by an enlargement of the ein before the obstruction and congestion and filling of the collateral circulation in the direction of the stomach, oesophagus, retroperitoneum, large intestine and the surroundings of the spleen. The collateral circulation was partly hepatofugal, partly hepatopetal and sometimes via this circulation the remainder of the patent portal vein below the obstruction was filled. The exact cause of the occlusion could not be revealed in all instances sometimes I was the pressure



Fig. 6. Inoperable tumour of body of pancreas. Complete occlusion of lienal vein in its central portion with displacement of its filled portion to the left. Extensive hepatopetal collateral circulation in gastric region and region of large intestine. The hepatofugal collaterals in the region of the cardia and lower part of the oesophagus are also filled.

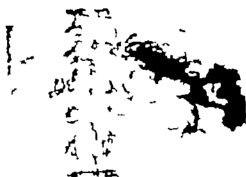


Fig. 7. Inoperable tumour of body and tail of pancreas. Complete occlusion of lienal vein closely after its origin. Ample collateral circulation to gastric region where there are varicose plexuses.



Fig 8. Inoperable tumour of tail of pancreas. Advanced occlusion of lienal vein in its left portion with collateral circulation to gastric region.



Fig 9 Postnecrotic pseudocyst of pancreas. Complete occlusion of lienal vein before spine Extensive hepatopetal collateral circulation via gastric veins, veins of large intestine and hepatocolic ligament. Hepatofugal collaterals in region of cardia, lower part of oesophagus and retroperitoneum

of the tumour sometimes its penetration into the veins and in three patients on necropsy a tumorous thrombosis was revealed

The X ray examination of the stomach and duodenum in patients where on the splenoportogram there were changes of the lienoportal trunk gave different results In two patients no changes were detected In three we found an unconvincing enlargement of the duodenal window and in six there were marked changes, particularly an enlargement of the duodenal window and pressure changes on the antrum of the stomach, sometimes associated with signs of penetration of the tumour

Inoperable tumours of the body of the pancreas

In patients with tumours of the body of the pancreas the tumour usually was also relative large, but only in five patients was it palpable. It manifested itself usually by typical clinical symptoms, pains, digestive disturbances and loss of weight.

Splenoportography revealed in these patients already more unequivocal changes than in tumours of the head of the pancreas. A normal picture was found only in one patient, where the tumour grew in a forward direction, was not detected by other examinations either and was found only on necropsy In another patient a slight narrowing of the lienal vein before the left edge of the spine was found with uneven outlines, due to the penetration of the tumour In the remaining ten patients a complete or almost complete occlusion of the lienal vein with all sequelae was found. The occlusion was localized in four patients at the left border of the spine in five in the median part of the lienal vein and in one patient already at the hilus of the spleen. The filled part of the lienal vein was sometimes deformed and sometimes it had irregular outlines. In these cases frequently the open portion of the portal vein and the hepatic branches were filled via the hepatopetal collaterals of the stomach and intestine. Tumorous thrombosis as the cause of the occlusion was found twice on necropsy

The X-ray examination of the stomach and duodenum revealed in two patients a normal finding, in three only unconvincing changes and in seven marked changes which suggested the presence of a tumour originating in the body of the pancreas.

Inoperable tumours of the tail of the pancreas

In both our patients the tumour was relatively large and penetrated into the surrounding tissue It was, however not palpable Clinically it manifested itself mainly by pain and loss of weight On examination of the stomach we found pressure changes on the posterior and lateral wall of the body of the stomach and an enlarged retrogastric space Splenoportography revealed in one patient a complete occlusion of the lienal vein already at the hilus of the spleen with a filling of the newly formed collaterals perisplenically and in the region of the great omentum. In the second patient we found a partial occlusion

of the lienal vein closely after its site of origin with marked circular stenosis and collateral circulation in the region of the gastric fundus, where the network of enlarged submucosal veins was filled.

Postnecrotic pseudocysts

The development and clinical manifestations of pseudocysts were typical in most instances. In eight patients the pseudocyst developed at different times following acute necrosis of the pancreas. Only in two patients did the necrosis not take an acute course and its symptoms were masked by symptoms of the primary affection of the gallbladder or liver. The pseudocyst was in most instances extensive and palpable as resistance of different size. On X-ray examination of the digestive tract pressure changes on the neighbouring organs were well marked. On operation, usually pancreatocystogastrostomy according to Jurasz, 1/2 to 2 litres of an opaque, usually reddish brown liquid were removed.

Before operation eight patients were examined splenoportographically and in three of these patients examinations were carried out repeatedly at different times after operation. The remaining two patients were examined only after operation. On examination before operation was found in one patient considerable pressure changes in the right half of the lienal vein. In another six patients splenoportography revealed complete occlusion of the lienal vein in the median portion with an extensive hepatopetal collateral circulation which bypassed the occlusion. Via the short gastric veins the arkedly enlarged submucosal gastric plexuses, the coronary vein, and from it the portal vein were filled, the latter being in some instances pressed to the right side to varying extent. The portal vein and its hepatic branches were also filled via the veins of the large intestine and the newly formed collaterals in the hepatocolic ligament. The occlusion was either due to pressure of the pseudocyst only or to an associated thrombosis which complicated the long persisting pseudocyst. This was revealed by control splenoportograms and on necropsy. In two patients who were operated on within two month after the acute necrosis, repeated splenoportography 6—18 months after operation revealed only partial occlusion. The lienal vein was deformed, narrowed — no doubt as result of adhesions from surrounding tissue but it allowed the passage of blood and the collateral circulation was also considerably less extensive. In third patient operated on only six months after acute necrosis of the pancreas, the splenoportographic finding did not change even after operation, complete occlusion of the lienal vein persisted which was certainly due to an associated thrombosis. Thrombosis was found by the pathologist also in two patients who were

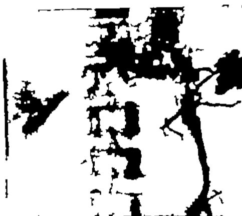


Fig. 10 Postnecrotic pseudocyst of pancreas. Complete occlusion of lienal vein in its central portion. Ample hepatoduodenal collateral circulation in gastric region where there are submucosal varices, and in the region of the large intestine.



Fig. 11 Status after operation of postnecrotic cyst of pancreas nine years ago. Lienal vein before spine is deformed, markedly narrowed with uneven outlines, most probably as result of parietal thrombosis. Small collaterals to retroperitoneum and stomach, marked splenomegaly.



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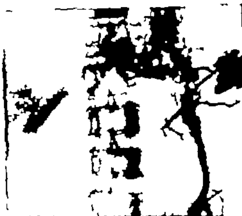


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veins, mainly the portal vein, and the amputated em usually has smooth outlines and sometimes a conical ending. An occlusion caused by pancreatitis is suggested by the presence of concrements or calcifications in the pancreas. In thrombosis the spleen is usually markedly enlarged. Usually it is, however not possible to detect exactly the origin of changes from the splenoportograms alone. If however the splenoportogram is evaluated in conjunction with the other examinations and the clinical picture, it is usually readily possible to find the cause of the changes and the nature of the pathological process.

Splenoportography however helps not only to detect a favorably localised tumour. It helps also to define its localization, its size, especially if a dorsally spreading tumour and its relation to the large veins. It thus helps the surgeon to determine the operability of the tumour and to plan the operation. If the tumour causes marked changes, particularly if it is already penetrating into the veins or causes their occlusion, the tumour is either inoperable, or in order to ensure its radical removal it is necessary to extend the operation also to neighbouring organs and tissues. On the other hand, if in a tumour detected by other methods the lienoportal trunk is not impaired — this implies mainly tumours of the papilla and head of the pancreas — or if only pressure changes are apparent on the eins, we may expect a typical radical operation to be feasible. Splenoportography moreover helps the surgeon also by detecting or ruling out metastatic foci in the liver. It must, however be emphasised, that splenoportography gives only limited help in determining the operability of tumours, and that splenoportographic results are only one of the factors which when evaluated comprehensively make it possible to determine the operability and select the most suitable surgical procedure.

A further partial contribution of splenoportography related to therapy is shown by our experience with pseudocysts. Though they are not numerous, they clearly show the importance of early operation. Pseudocysts cause considerable changes of the portal system, particularly occlusion of the lienal vein and a considerable varicose enlargement of the eins, particularly those of the stomach and large intestine. At first the occlusion is due merely to pressure and thus if the operation is carried out in time, and the pseudocyst is emptied, the passage through the lienal vein is renewed and the collateral circulation disappears. If, however the operation is not carried out in time, the occlusion due to pressure is complicated by thrombosis and the patient is threatened by all its sequelae. In operations of long persisting pseudocysts it is therefore necessary to consider also simultaneous splenectomy.

The possibilities and the sensitivity of splenoportography are apparent also from a comparison of splenoportographic pictures and findings of examination of the stomach and duodenum which are most frequently used in the diagnosis of pancreatic tumours. This comparison indicates clearly the advantage of splenoportography. Thus in 36 patients where splenoportography revealed marked changes of the lienoportal trunk, the results of the examination of the stomach nd duodenum were five times normal, eight times there were unconvincing changes and in 23 patients there were evident changes. Moreover splenoportography has, as compared with other examinations the dvantage that the

operated on more than six months after acute necrosis and who died after operation

In the last patient examined before operation, the contrast substance remained during twice repeated splenoportography in the parenchyma of the spleen, and neither the portal circulation nor the collateral circulation were filled. In our opinion this is due to a complete block of the arterial and venous circulation by a pseudocyst. The remaining two patients were examined only after operation which was performed seven and nine years ago resp. Splenoportography in these patients revealed a deformation of the right half of the lienal vein and its narrowing with irregular defects which aroused suspicion of a parietal thrombosis. The collateral circulation in these patients was only slight and only the short gastric vein was filled.

Discussion

Our above described experience agrees on the whole with that of other authors and confirms the possible value of splenoportography in the diagnosis of tumours of the pancreas. The survey shows that the use of splenoportography is a valuable contribution. Yet it is clear that even this method of examination will not solve the problem of the diagnosis of pancreatic tumours completely as it also has its diagnostic limitations and shortcomings Like most other methods of examination it does not detect the tumour directly but only from secondary changes caused by the tumour on the lienoportal trunk. Its diagnostic possibilities are therefore determined by the relationship of the tumour to the veins of this trunk and in particular by the site of the tumour its size and the direction of its growth Splenoportography thus can detect very easily a tumour growing from the posterior parts of the pancreas and spreading in a dorsal direction, particularly tumours of the body and tail because these parts of the pancreas are closest to the lienal vein In these instances splenoportography can reveal already very small tumours, as shown by Leger In his patient only a repeated operation focussed on the site of changes ascertained on the splenoportogram confirmed a tumour of the tail of the pancreas. On the other hand, splenoportography is unable to detect even a larger tumour which has no close relationship to the lienoportal trunk. Most frequently these are tumours growing from the region surrounding the papilla of Vater and the head of the pancreas, but it may even be a tumour of the body or tail of the pancreas growing in a ventral direction For detection of the pancreatic tumour the pathological finding alone is thus decisive

Another shortcoming of splenoportography is the fact that there do not exist changes specific only for tumours of the pancreas. Similar changes may be due also to tumours in other organs, as well as to inflammatory processes or even primary affections of the veins. In the differential diagnosis account must be taken mainly of gastric tumours, and tumours of the large intestine and of the gallbladder spreading dorsally and penetrating into the veins, as well as renal tumours and tumours of the retroperitoneum metastases in the parapancreatic and paraaortal nodes, chronic hypertrophic pancreatitis especially if localised post inflammatory adhesions, and also thrombosis of the lienal and portal vein. On the splenoportogram, particularly in the filled portions of the affected veins, changes are often observed which make it possible to draw some conclusions also on the nature of the pathological process Thus the presence of malignant tumours is suggested by uneven dentated outlines of the veins indicating direct penetration Pseudocysts on the other hand often cause pressure changes on the filled

veins, mainly the portal vein, and the amputated vein usually has smooth outlines and sometimes conical ending. An occlusion caused by pancreatitis is suggested by the presence of concrements or calcifications in the pancreas. In thrombosis the spleen is usually markedly enlarged. Usually it is, however, not possible to detect exactly the origin of changes from the splenoportograms alone. If however the splenoportogram is evaluated in conjunction with the other examinations and the clinical picture it is usually readily possible to find the cause of the changes and the nature of the pathological process.

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changes in the filled veins are usually readily recognised easily evaluated and are objective. In examinations of the stomach and duodenum the evaluation of unconvincing changes is very subjective and depends on the examining physician We do not mean to say however that splenoportography can replace these examinations These will remain in future fundamental examinations, and splenoportography will supplement them when they are negative or uncertain or when the results are evident but we wish to know more details about the tumour Problems of accurate and early diagnosis of tumours of the pancreas and their detection in the operable stage do not depend only on the sensitivity of the examination methods such as splenoportography The greatest difficulties in this respect are caused by the very late occurrence of subjective and objective symptoms. Particularly tumours of the body and tail but sometimes also tumours of the head take at first a latent course or are associated only with mild symptoms. When the symptoms attain an intensity such that they arrouse suspicion of a tumour it is usually too late and the tumour is usually inoperable Early diagnosis thus can be helped particularly if the possible presence of a tumour is considered even if the patient has undefined complaints and when in addition to other examinations a splenoportographic examination is made. From the above described diagnostic limitations it appears that splenoportography does not detect every tumour but that it can detect even small ones particularly favorably localised tumours of the body and tail of the pancreas at a stage so early as to have escaped attention and can thusrender a successful surgical operation possible.

Conclusion

Splenoportography is a valuable contribution to the diagnosis of tumours of the pancreas. It helps to fill the gaps in hitherto used methods of examination, and in view of its diagnostic possibilities it must be included among the main methods Its greatest contribution is the improvement of the diagnosis of tumours of the body and tail of the pancreas. In the diagnosis of tumours of the head of the pancreas its possibilities are more limited Splenoportography is also very valuable for assessing the operability of tumours. The basic prerequisite for a useful outcome of the examination is the comprehensive evaluation of splenoportograms in conjunction with the clinical picture and other methods of examination.

Summary

After drawing attention to the requisite conditions for the use of splenoportography in the diagnosis of diseases of the pancreas the authors present a brief account of the world literature and submit their own experience with tumours of the pancreas. They give an account of their own technique of examination, and of the normal picture, and summarise changes which they demonstrated in 34 patients with malignant tumours and in 10 patients with postnecrotic pseudocysts In analysing their own experience they draw attention to the advantages and shortcomings of splenoportography they define the limits of its diagnostic possibilities and evaluate its help in determining the operability of a tumour and in planning the operation. Next they compare the splenoportographic findings with those of peroral examinations of the stomach and duodenum and draw at

tention to the prerequisites and possibilities for early diagnosis of tumours of the pancreas. In the conclusion they evaluate the role of splenoportography in this diagnosis and emphasise the need for a comprehensive evaluation of splenoportography.

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Contribution of Splenoportography to the Diagnosis of Diseases of the Pancreas

II. Inflammatory Diseases

By

Josef Rosch and Karel Herfort

In the diagnosis of inflammatory diseases of the pancreas splenoportography has hitherto been used only rarely since for a long time pancreatitis was not, and frequently still is not considered an indication for splenoportography. When patients with inflammatory affections of the pancreas were subjected to splenoportography the indication was usually some sign of portal hypertension, most frequently haemorrhage into the gastrointestinal tract. These patients suffered from an ad anced stage of the disease, which, however in some instances took latent course where the pancreas with the inflammatory changes caused more extensive hanges n th portal system, particularly segmental portal hypertension. Splenoportography revealed in these patients different degrees of venous occlusion usually advanced or complete occlusion of the lienal vein with all its consequences.

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More systematic examinations of patients with nflammatory diseases of the pancreas were nitiated onl by Leger and Caroli and Hepp ga e also an account of thei experience. In various stages of the disease they encountered only rarely a normal picture. They usually found a slightly increased portal pressure, dilatation of the lienal vein and frequently also arious degrees of compression of the lienal vein before the spine, from a slight deformation up to occlusion w th complete stenosis.

At first we did not examine our patients suffering from inflammatory diseases of the pancreas by splenoportography. We focused our ttention to these patients only in 1955 when we found in a patient with cholelithiasis a deformation of the lienal vein and congestion the cause of which — a pancreas enlarged due to inflammation — was revealed only on operation. In the present communication



Fig 1 Chronic relapsing pancreatitis. Grade I changes. Mild narrowing of lienal vein before spine.



Fig 3 Chronic hepatitis. Chronic pancreatitis Grade I changes. Slight localised reduction of contrast filling before left half of spine



Fig 2 Chronic relapsing pancreatitis. Grade I changes. Narrowing of lienal vein before spine

we shall evaluate, based on reports in the literature and our own experience the contribution and possibilities of sple noportography in the diagnosis of inflammatory diseases of the pancreas.

Material and technique

Ninety five patients, most of them suffering from chronic relapsing pancreatitis, were examined. They included 61 men (64.2 per cent) and 34 women (35 8 per cent) The average age of the patients was 46 years. By chronic relapsing pancreatitis we mean an affection of the external secretory portion of the pancreas characterised by relapses of sublethal attacks of acute pancreatitis or acute interstitial inflammations of Elman's type. Such attacks and their morphological consequences are the basis of the clinical picture of chronic relapsing pancreatitis. Chronic relapsing pancreatitis was diagnosed in the quiescent stage from the clinical picture and functional examination of the external secretion of the pancreas by secretin tests, in the acute stage from the clinical picture and amylase values, and in some patients serum lipase. In 27 patients (28.4 per cent) the diagnosis was confirmed moreover on operation and/or by biopsy In all patients the disease manifested itself by typical clinical symptoms which served as an indication for splenoportography The changes ascertained on splenoportography in these patients served as a basis for analysis and for assessing changes typical of inflammatory affections of the pancreas.

The technique used for examination was the same as in patients with tumours affections of the pancreas (see part I of the present paper)

Results

According to the degree and extent of changes on the splenoportal trunk we divided the 95 patients with chronic relapsing pancreatitis into four groups.

I The first group included six patients (6 3 per cent) where splenoportography revealed a normal picture the splenoportal trunk was not affected These patients had

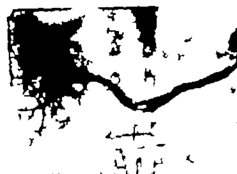


Fig. 4 a.



Fig. 4 b.

Fig. 4. Chronic relapsing pancreatitis. Grade II changes.

) On injection of contrast material. Picture of pelotte with more marked localized dilatation of lienal vein before left margin of spine.

b) Fifteen seconds after injection. Persistence of contrast material in lienal vein with simultaneous marked opacity of liver



Fig. 5 a.

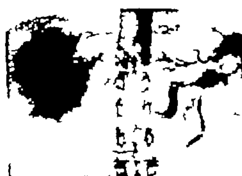


Fig. 5 b.

Fig. 5. Chronic relapsing pancreatitis. Cholelithiasis. Grade II changes with more advanced congestion.

) On injection of contrast substance. Deformation and narrowing of lienal ein before lef border of spine. Retrograde filling of lower mesenteric ein and short gastric veins.

b Ten seconds after injection. Persistence of contrast substance in lienal vein and in its afferent branches

several relapses of the disease and at the time of examination the disease was quiescent. The X-ra examination of the stomach and duodenum did not reveal any substantial changes in these patients. I one of the patients who was operated on, the surgeon found normal-sized pancreas which was merely tougher.

In the second group 17 patients (17.9 per ent) were included where slight deformation of the lienal vein before the spine was found. The median portion of the lienal ein, usually before the left half of the spine, was either slightly narrowed and flattened at the bottom, or on the contrary slightly dilated, its filling then being less contrasting. The portion of the lienal ein on the left side of the spine was frequently wider and homogeneously filled. The emptying of the lienal ein was not impaired and was always normal. These changes were termed *grade I changes*.

The patients in this group were examined during various stages of the disease. Six patients were examined during relapse of pan-



Fig 6 a.



Fig 6 b.

Fig 6 Chronic hepatitis. Chronic pancreatitis. Grade II changes with advanced congestion.
a) On injection of contrast substance Marked dilatation of lienal vein and portal vein. Retrograde filling of lower mesenteric vein, short gastric veins and hilar branches of spleen.
b) Eight seconds after injection. Persistence of contrast substance in lienal vein and in its filled afferent branches.

creatitis, the remainder during the quiescent stage of the disease The X ray examination of the stomach and duodenum revealed in these patients a normal picture or only undefined and inconvincing changes on the duodenum. Four patients were operated on and the surgeon found a mild usually diffuse inflammatory enlargement of the pancreas.

3 The third and most numerous group is formed by 68 patients (71 4 per cent) where splenoportography revealed typical deviations which were termed ***grade II changes*** by the authors. When injecting the contrast material, the lienal vein on the left of the spine was usually slightly dilated homogeneously and amply filled and sometimes its afferent branches were filled from it in a retrograde fashion. These included most frequently the hilar branches of parts of the spleen not visualized by contrast, the short gastric veins and the lower mesenteric vein Before the spine and again most frequently before its left half the lienal vein was deformed in different ways, as in grade I changes. Sometimes the lienal vein was narrowed at these sites and its lower border flattened while in other instances it was dilated, less contrasting at these sites and slightly severed or knee shaped. On injection sometimes these deformities of the lienal vein before the spine were rather less marked. Quite marked changes, characteristic for grade II were apparent after completion of the injection. The contrast substance in the lienal vein became congested and persisted there for 10 20 and frequently more seconds. The portal vein and the hepatic branches, on the other hand, were emptied normally and on X-ray pictures taken later a homogeneous filling of the lienal vein on the left of the spine could be seen, simultaneously with opacity of the liver On the late pictures the lienal vein was in some instances wider than on injection and as slightly dilated. The contrast substance was then usually drained slowly into the portal vein and also the afferent branches of the lienal vein were drained in the direction of the portal flow In several patients a few seconds after injection, most frequently 5—10 seconds, the lumen of the lienal vein was reduced, no doubt due to a spasm. The contrast substance passed in these instances to the afferent branches against the current and persisted for a long time in the lienal vein.

In five patients (7 4 per cent) splenoportography was made twice at different intervals from five month to two years. The splenoportographic pictures were either identical or revealed slight deviations as regards the degree of deformation of the lienal vein depending on the stage of the disease when the patients were examined. In the acute phase the changes were more marked, in the quiescent stage the regression of changes was apparent.

Most patients in this group had at the time of examination pronounced complaints due to relapse of the acute stage of the disease. In twelve patients (17.6 per cent) on X-ray examination of the stomach and duodenum marked enlargement of the duodenal window was found, in 28 patients (41.2 per cent) the changes were not convincing and in 28 patients (41.2 per cent) these examinations revealed a normal picture. In 17 patients subjected to operation the surgeon found various degrees of enlargement of the pancreas, which was either diffuse or affected predominantly at the head. Only in two patients, where the interval between operation and splenoportography was longer and the disease was already in quiescent stage at the time of operation, did the operation not reveal substantial enlargement of the pancreas.

4. The fourth group is formed by four patients (4.2 per cent) who differed from the rest by their splenoportographic as well as anatomical finding. All these patients had suffered from several severe relapses of the disease and in all there were marked or suspect parenchymatous calcifications on the pancreas. In three patients who were operated on the surgeon found an uneven enlargement of the body of the pancreas which changes in the surrounding tissues.

Splenoportography in these patients revealed extensive changes which were termed *grade III changes*, and which are manifested by an advanced degree of venous occlusion. The medium portion of the lienal vein on the left of the spine was considerably deformed, markedly narrowed or even completely interrupted, and more extensive collateral circulation became filled. In two patients the occlusion was incomplete, in two complete.

The X-ray examination of the stomach and duodenum revealed marked changes suggesting enlargement of the pancreas only in two patients.



Fig. 7. Chronic relapsing pancreatitis with localised enlargement of body. Susp. calcification in pancreas. Grade III changes. Advanced occlusion of lienal vein in its central part with marked deformation. Hepatopetal collateral circulation via short gastric veins, submucous gastric veins and via coronary veins of stomach.

Discussion

As in tumours of the pancreas, the revealed changes in patients with chronic pancreatitis are in agreement with reports in the literature and jointly suggest the possibilities of splenoportography in the diagnosis of inflammatory diseases of the pancreas. Splenoportography here proves to be a sensitive method which renders early and reliable diagnosis possible, as the pancreas altered by inflammation particularly in becoming enlarged causes changes on the splenoportal trunk, particularly on the lienal vein, frequently very early ones. The most important part in the development of these changes is played by the direct pressure of the enlarged pancreas, which may be supplemented by secondary changes, such as traction or pressure due to adhesions, venous spasms or even complications of pancreatitis, particularly secondary thrombosis of the lienal vein or portal vein. Pressure changes are found more frequently and also earliest in pancreatitis. Their localisation, character and extent differ depending on the part of the pancreas affected and the degree of enlargement.

In diffuse enlargement of the pancreas or if the head is affected predominantly changes on the internal portion of the

lienal vein before the spine become most marked. There the lienal vein cannot yield to the pressure of the enlarged pancreas because it is pressed by it against the spine and large vessels and is thus soon deformed Changes of this portion of the vein develop very early are readily evaluated and are a reliable diagnostic sign. According to their degree, and to render them more instructive they were divided into three groups. This classification is however only schematic the differences between different degrees being only quantitative, not sharply defined and overlapping

When the pancreas is only slightly enlarged, splenoportography reveals grade I changes with manifestations depending on the direction of the pressure. If the pressure acts more from the bottom, the lower border of the lienal vein before the spine becomes flattened and narrowed If the pressure acts from a frontal direction the picture of a "pelotte develops at these sites — a reduction of the contrasting properties of the filling and frequently also an enlargement of this portion of the vein. The extent of these changes may vary but it must be emphasized that the changes particularly in the initial stages, must not be confused with the picture of dilution of the contrast substance at the site of the inflow of non contrasting blood from the upper mesenteric vein. The reduced depth of the filling is in the latter case more marked on the right side and proceeds into the portal vein. When the enlarged pancreas exerts a pressure, the changes in the depth of the filling are defined and frequently localised before the left half of the spine. The remaining portions of the lienal vein on the left of the spine are frequently wider due to the more difficult passage and slight degree of passive portal hypertension Marked changes in the evacuation are, however not encountered.

If the enlargement of the pancreas is more extensive and the pressure against the spine greater grade II changes develop with marked congestion in the lienal vein which is characteristic for these changes. On injection again a deformation of the lienal vein before the spine is apparent and a dilatation of the portion of the lienal vein on the left from the spine. Moreover frequently as a sign of a more advanced passive portal hypertension and congestion the afferent branches of the lienal vein are filled. The deformation of the vein before the spine need however not be always clearly visible during injection and may be visualized only after injection. This is due to the fact that during injection of the contrast substance into the hilar portion of the spleen the pressure of the injection is transmitted to the portal circulation and acts thus against the pressure of the enlarged pancreas. After the injection has been completed the pressure in the circulation declines to the previous level the pressure of the enlarged pancreas predominates and the deformation is more marked and particularly the congestion in the lienal vein is more pronounced The picture of the very slow emptying of the lienal vein is, however certainly influenced by the fact that when the blood rate is reduced the heavier contrast substance sinks to the lower portions of the vein where it persists for a long time. It is drained usually only after a change of posture of the patient or after a change of pressure in the circulation, for instance during deep respiration The picture of grade II changes, and particularly the sign of the persistence of contrast substance in the lienal vein, must not be confused with the remaining

filling in this vein which sometimes is found if the remnant of contrast substance in the spleen is greater. In the latter case the filling is never homogeneous and forms only thin and poorly contrasting strips, particularly near the wall of the vein.

With the gradual enlargement of the pancreas the pressure deformation of the lienal vein before the spine also encreases, as well as the congestion, and grade III changes develop which are manifested by an already advanced degree of venous occlusion. These changes can be due to pressure only but frequently an important part is played by associated thrombosis. The lienal vein is usually very narrowed before the spine, sometimes completely severed — amputated, and then even on injection no contrast material passes through the site of occlusion. If the occlusion is due to mere pressure the outlines of the deformed vein are usually smooth and sharp. In grade III changes the contrast substance fills an extensive collateral circulation which is usually of the hepatopetal type; frequently however simultaneously hepatofugal collaterals are found. Most frequently there is retrograde filling of the typical afferent branch of the lienal vein, particularly the short gastric veins from which in the region of the fundus and corpus of the stomach ample submucous plexuses are formed, and the contrast substance passes from these plexuses through the coronary vein of the stomach to the portal vein. A similar hepatopetal route, which is frequently encountered in complete occlusion and which by-passes the latter, is formed by the veins of the large intestine, particularly the veins of the transverse colon. In prolonged occlusion, and particularly in the case of secondary inflammatory changes in the tissues surrounding the pancreas or in secondary thrombosis, usually new collaterals develop to the neighbouring organs and tissues, particularly in adhesions.

If the enlargement affects only the body or tail of the pancreas, early diagnosis is more difficult. The lienal vein has then a certain though limited liability to shift and can to a certain extent yield to the pressure of the enlarged pancreas. Moreover the evaluation of the course and configuration of the lienal vein is then rendered more difficult, because its range of variation at these sites is considerable, even under normal conditions. We may therefore classify as pressure changes only those deformations and abnormal configurations of the lienal vein where changes in the shape and course are associated with changes in the filling or lumen and perhaps also in drainage. However, a smaller localised enlargement of the body or tail of the pancreas, unless the parenchyma in the close vicinity of the lienal vein is affected, cannot be so readily diagnosed and only more extensive localised enlargement of this type can be diagnosed by splenoportography. On the lienal vein changes of different degrees may be again encountered, depending on the extent of enlargement. Grade I and II changes are, however, in view of the above circumstances less readily detectable, and only rarely will it be possible to prove them. Usually in this type of enlargement of the pancreas only grade III changes are encountered.

Secondary changes are encountered less frequently than pressure changes. Traction or pressure of adhesions is found most frequently in inflammatory changes of tissues surrounding the pancreas, either when the inflammation spreads to surrounding tissues or if there are changes

lienal vein before the spine become most marked. There the lienal vein cannot yield to the pressure of the enlarged pancreas, because it is pressed by it against the spine and large vessels and is thus soon deformed. Changes of this portion of the vein develop very early are readily evaluated and are a reliable diagnostic sign. According to their degree, and to render them more instructive, they were divided into three groups This classification is, however only schematic, the differences between different degrees being only quantitative, not sharply defined and overlapping

When the pancreas is only slightly enlarged, splenoportography reveals grade I changes with manifestations depending on the direction of the pressure If the pressure acts more from the bottom, the lower border of the lienal vein before the spine becomes flattened and narrowed If the pressure acts from a frontal direction the picture of a "pelotte develops at these sites — a reduction of the contrasting properties of the filling and frequently also an enlargement of this portion of the vein. The extent of these changes may vary but it must be emphasized that the changes particularly in the initial stages must not be confused with the picture of dilution of the contrast substance at the site of the inflow of non contrasting blood from the upper mesenteric vein. The reduced depth of the filling is in the latter case more marked on the right side and proceeds into the portal vein. When the enlarged pancreas exerts a pressure, the changes in the depth of the filling are defined and frequently localised before the left half of the spine. The remaining portions of the lienal vein on the left of the spine are frequently wider due to the more difficult passage and slight degree of passive portal hypertension. Marked changes in the evacuation are, however not encountered.

If the enlargement of the pancreas is more extensive and the pressure against the spine greater grade II changes develop with marked congestion in the lienal vein which is characteristic for these changes. On injection again a deformation of the lienal vein before the spine is apparent and a dilatation of the portion of the lienal vein on the left from the spine. Moreover frequently as a sign of a more advanced passive portal hypertension and congestion the afferent branches of the lienal vein are filled. The deformation of the vein before the spine need however not be always clearly visible during injection and may be visualized only after injection This is due to the fact that during injection of the contrast substance into the hilar portion of the spleen the pressure of the injection is transmitted to the portal circulation and acts thus against the pressure of the enlarged pancreas. After the injection has been completed the pressure in the circulation declines to the previous level the pressure of the enlarged pancreas predominates and the deformation is more marked and particularly the congestion in the lienal vein is more pronounced. The picture of the very slow emptying of the lienal vein is, however certainly influenced by the fact that when the blood rate is reduced the heavier contrast substance sinks to the lower portions of the vein where it persists for a long time It is drained usually only after a change of posture of the patient or after a change of pressure in the circulation, for instance during deep respiration. The picture of grade II changes, and particularly the sign of the persistence of contrast substance in the lienal vein, must not be confused with the remaining

filling in this vein which sometimes is found if the remnant of contrast substance in the spleen is greater. In the latter case the filling is never homogeneous and forms only thin and poorly contrasting strips, particularly near the wall of the vein.

With the gradual enlargement of the pancreas the pressure deformation of the lienal vein before the spine also increases, as well as the congestion, and grade III changes develop which are manifested by an already advanced degree of venous occlusion. These changes can be due to pressure only but frequently an important part is played by associated thrombosis. The lienal vein is usually very narrowed before the spine, sometimes completely severed — amputated, and then even on injection no contrast material passes through the site of occlusion. If the occlusion is due to mere pressure the outlines of the deformed vein are usually smooth and sharp. In grade III changes the contrast substance fills an extensive collateral circulation which is usually of the hepatopetal type frequently however simultaneously hepatofugal collaterals are found. Most frequently there is retrograde filling of the typical afferent branch of the lienal vein, particularly the short gastric veins from which in the region of the fundus and corpus of the stomach ample submucous plexuses are formed, and the contrast substance passes from these plexuses through the coronary vein of the stomach to the portal vein. A similar hepatopetal route, which is frequently encountered in complete occlusion and which by passes the latter is formed by the veins of the large intestine, particularly the veins of the transverse colon. In prolonged occlusion, and particularly in the case of secondary inflammatory changes *in the* tissues surrounding the pancreas or in secondary thrombosis, usually new collaterals develop to the neighbouring organs and tissues, particularly in adhesions.

If the enlargement affects only the body or tail of the pancreas, early diagnosis is more difficult. The lienal vein has then a certain though limited liability to shift and can to a certain extent yield to the pressure of the enlarged pancreas. Moreover the evaluation of the course and configuration of the lienal vein is then rendered more difficult, because its range of variation at these sites is considerable, even under normal conditions. We may therefore classify as pressure changes only those deformations and abnormal configurations of the lienal vein where changes in the shape and course are associated with changes in the filling or lumen and perhaps also in drainage. However a smaller localised enlargement of the body or tail of the pancreas, unless the parenchyma in the close vicinity of the lienal vein is affected cannot be so readily diagnosed, and only more extensive localised enlargement of this type can be diagnosed by splenoportography. On the lienal vein changes of different degrees may be again encountered, depending on the extent of enlargement. Grade I and II changes are, however in view of the above circumstances less readily detectable, and only rarely will it be possible to prove them. Usually in this type of enlargement of the pancreas only grade III changes are encountered.

Secondary changes are encountered less frequently than pressure changes. Traction or pressure of adhesions is found most frequently in inflammatory changes of tissues surrounding the pancreas, either when the inflammation spreads to surrounding tissues or if there are changes

in the nodes. The lienal vein is then usually severed and bent. This type of deformation was observed several times in the portion before the spine. A similar deformation with a sharp bend of the trunk at the site of the confluence of the lienal and portal vein may however also be due to pressure in localised enlargement of the pancreas, which acts on the inner third of the lienal vein the latter takes an upward course to the right whereby the sharp bend is at the orifice of the upper mesenteric vein

The presence of spasms, as encountered several times, is easily understandable, particularly during relapses of pancreatitis and in the acute or subacute stage of the disease. Their participation in the development of changes on the splenoportal trunk cannot, however be readily evaluated as yet.

Thrombosis of the lienal vein and in particular of the portal vein in chronic pancreatitis is a relatively rare complication. It is encountered most frequently in patients with a severe course of the disease which manifests itself by severe attacks or even by necroses There the inflammation spreads frequently to the neighbouring tissues the lienal vein may be also affected and secondary thrombophlebitis develops The thrombosis may be only partial, but more frequently it fills the entire lumen of the vein. On the splenoportogram grade III changes are then apparent with an extensive collateral circulation whereby frequently also newly formed veins are filled The outlines of the veins in thrombosis are often blurred and uneven, and the diagnosis of thrombosis as the cause of occlusion can be also facilitated by the size of the spleen which is usually larger than in occlusion due to mere pressure The precise assessment of the cause of occlusion from the splenoportogram only is however usually not readily possible

The value of the occurrence and magnitude of the above changes on the splenoportal trunk for the diagnosis of pancreatitis is variable. All changes have only a local character and it cannot be stated that they are specific for inflammatory disease of the pancreas only Nevertheless with some changes pancreatitis and enlargement of the organ may be anticipated merely from the splenoportogram with high probability For other changes however it is impossible to draw any conclusion on the nature of the disease from the splenoportogram or even to decide whether the pancreas or another organ or tissue of the epigastrium is affected. Nevertheless when the splenoportograms are evaluated in conjunction with other examinations and the general clinical picture, it is usually possible to establish the diagnosis very accurately and it is possible to detect details on the pancreas itself The basic prerequisite for a correct evaluation of splenoportography is therefore, as for tumours of the pancreas and affections of other organs, namely a comprehensive evaluation of splenoportograms.

With grade I changes there can be only a suspicion of pancreatitis with a slight enlargement of the organ in those instances where the deformation of the lienal vein is before the spine and where it is clearly marked. In these instances it is, however not possible to conclude for certain that pancreatitis is present because grade I changes caused by pancreatic enlargement due to inflammatory changes cannot always be reliably differentiated from changes due to smaller operable tumours localised in the head of the pancreas. Grade I changes can

usually be differentiated from pressure changes caused by metastatically enlarged peripancreatic nodes, as the former are found on the lower border of the vein or have the character of a pelott When the nodes are enlarged, the changes are mostly encountered on the upper border line of the splenoportal trunk and usually there are several foci. It is even more difficult to evaluate the cause of defor mation of the vein as regards grade I changes if the latter are found in the por tion of the lienal vein on the left of the spine. In the differential diagnosis of these cases, apart from other possibilities tumours of the retroperitoneum must be considered, as well as tumours of the left kidney and without a detailed knowledge of the clinical picture the cause of the changes cannot be revealed.

It is equally difficult to diagnose the causes of grad III changes. These hanges are encountered less frequently pancreatitis than in tumours of the pancreas, postnecrotic pseudocysts, gastric tumours penetrating dorsally or af fections of the veins alone, e. g thrombosis of the lienal vein or portal vein. An inflammatory origin of grade III changes can be assumed w th reasonable proba bility only when intraparenchymatous calcifications or concrements are found in the pancreas. Otherwise the causes of hanges can be detected only with the help of the clinical picture and other ex aminations.

Only grade II changes can be considered clea evidence of pancreatitis. They are considered typical for an inflammatory enlargement of the pancreas, either permanent or temporary due to oedema during relapses of the disease. W assume that its mechanical and func tional causes can hardly simulate other pathological processes. We are led to this belief by the fact that we did not encounter typical manifestations of grade II changes in any disease other than chronic pancreatitis. The fact that the surgeon did not find on operation of the two patients with these changes a marked enlargement of the pancreas may be due to the disease passing into the quiescent stage, and to the regression of oedema which was present during the period of the relapse of pancreatitis when the pa tient was subjected to splenoportographic examination. From grade II changes we deduced several times correctly the inflammatory nature of the disease, in cases where on operation the surgeon considered the enlargement of the pancreas erroneously as tumours or where the general finding suggested rather a malignant nature of the disease It must, however be emphasised that the presence of these changes does not enable one to rule out a small tumour of the pancreas which grows in the organ altered by in flammation or itself contributes to the development of secondary inflammatory changes. We were convinced of this by our two patients where a small tumour of the head alone did not cause any changes of the splenoportal trunk. When evaluat ng splenoportograms t is, of course, necessary to distinguish changes caused by the penetration of tumours into eins which on superficial examination may imitate grade II changes. We observed those several times in gastri tumours penetrating dorsally into the pancreas nd the area of the large veins. In these patients on injection a narrowing of the lienal vein before the spine was found and after completion of the injection the contrast substance also persisted in the lienal vein. The outlines of the narrowed site of the ein were however uneven or ill-defined. A differentiation from grade

II changes, where even a markedly deformed vein has sharp and smooth outlines did not cause any difficulties

Conclusion

Splenoportography helps also in the diagnosis of inflammatory conditions of the pancreas, and in view of its possibilities it must be included among the main methods of examination for these affections of the pancreas also as for tumours. It helps to detect pancreatitis associated with an enlargement of the organ and above all to diagnose pancreatitis where the enlargement affects diffusely the whole organ or its head In the diagnosis of pancreatitis with a localized enlargement affecting the body or tail of the pancreas, it proves a less sensitive method and helps to detect only more advanced enlargement. It is most convincing with grade II changes With changes of other grades the presence of pancreatitis may be assumed only in conjunction with the clinical finding and after ruling out processes which may cause similar changes

Summary

After a review of the world literature the authors give an account of their own experience based on the examination of 95 patients with inflammatory affections of the pancreas. The changes revealed by splenoportography in these diseases are divided according to their extent into three grades corresponding to the extent of the enlargement of the pancreas. In the analysis of their experience the authors indicate the causes and mechanisms of the development of the ascertained changes and their grades, they draw attention to processes and pictures which must be considered in the differential diagnosis and evaluate the diagnostic value of individual grades. In the conclusion they summarize the contribution of splenoportography to the diagnosis of inflammatory affections of the pancreas.

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The Small Intestine Transit Time in Steatorrhoea

By

Gunvor Persson and Olle Mattsson

Considerable variations in the transit time obtained with ordinary follow through examinations of the small intestine and the absence of any reliable relationship between results and clinical conditions have hardly stimulated the use of such examinations. Pygott (1958) for instance has the opinion that "routine requests for barium meal and follow through have no justification". As regards patients with steatorrhoea Brown (1959) claims, of 15 cases of suspected steatorrhoea, 3 were later unsubstantiated, and 10 are regarded on clinical grounds as having steatorrhoea. Of these only 2 were picked up at follow through, and 8 were reported normal. These statements are based on radiographical routine barium examinations of the small intestine with ordinary barium contrast media and probably structural integrity and mucosal pattern has been taken more into consideration than the transit time. "The danger lies in the common assumption that the rate of passage of food will be similar to that observed with barium" (Brown 1959).

In a method recently described (Lagerlöf, Mattsson and Persson 1959 and 1960) the usual barium-water mixture is replaced by a meal prepared of corn oil, skimmed milk powder, dextrose and water mixed with a special suspension stable barium sulphate preparation. The composition of the nutritive component is shown in table I.

This nutritive component was used by Borgström et al. (195) in a study on intestinal digestion and absorption.

The present paper is a study of the small intestine transit time of the above-mentioned food-barium meal in a group of patients with steatorrhoea as compared with that of normals.

Method

The procedure of examination has been exactly the same for all the patients in question and a special department has been reserved for the examinations so that these could proceed without disturbances from the routine work. All examinations started early in the morning with the patients on a fasting stomach. The barium-food mixture, which is

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II changes where even a markedly deformed vein has sharp and smooth out lines, did not cause any difficulties.

Conclusion

Splenoportography helps also in the diagnosis of inflammatory conditions of the pancreas and in view of its possibilities it must be included among the main methods of examination for these affections of the pancreas also as for tumours. It helps to detect pancreatitis associated with an enlargement of the organ and above all to diagnose pancreatitis where the enlargement affects diffusely the whole organ or its head. In the diagnosis of pancreatitis with a localised enlargement affecting the body or tail of the pancreas, it proves a less sensitive method and helps to detect only more advanced enlargement. It is most convincing with grade II changes. With changes of other grades the presence of pancreatitis may be assumed only in conjunction with the clinical finding and after ruling out processes which may cause similar changes.

Summary

After a review of the world literature the authors give an account of their own experience based on the examination of 95 patients with inflammatory affections of the pancreas. The changes revealed by splenoportography in these diseases are divided according to their extent into three grades, corresponding to the extent of the enlargement of the pancreas. In the analysis of their experience the authors indicate the causes and mechanisms of the development of the ascertained changes and their grades; they draw attention to processes and pictures which must be considered in the differential diagnosis and evaluate the diagnostic value of individual grades. In the conclusion they summarize the contribution of splenoportography to the diagnosis of inflammatory affections of the pancreas.

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The Small Intestine Transit Time in Steatorrhoea

By

Gunvor Perman and Ove Mattsson

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This nutritive component was used by Borgström et al. 1957 in a study on intestinal digestion and absorption.

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The procedure of examination has been exactly the same for all the patients in question and a special department has been reserved for the examinations so that these could proceed without disturbances from the routine work. All examinations started early in the morning with the patients on a fasting stomach. The barium-food mixture which is

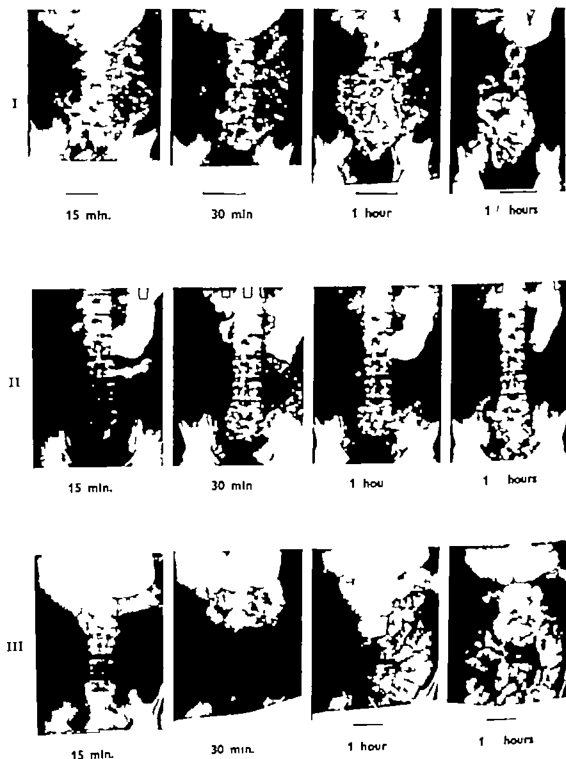


Fig 1 Comparison between th normal d the two pathological series regarding the transit up t 1 1/2 hours. Note the difference in filling of the intestines I Healthy student. II Case I in table IV III Case 5 in table III

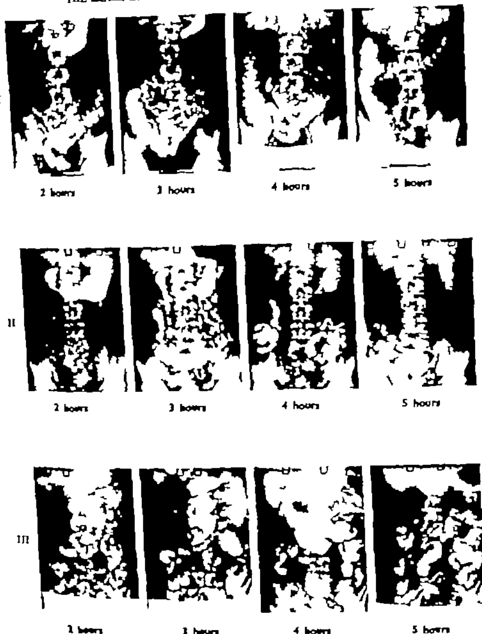


Fig. 2. Exposures taken between 2 and 5 hours after ingestion of the contrast-food meal in the normal and the two pathological groups. Differences in degree of filling are observed. The transit of the small intestine in the normal case is practically completed. I Healthy student. II Case 1 in table IV. III Case 5 in table III.

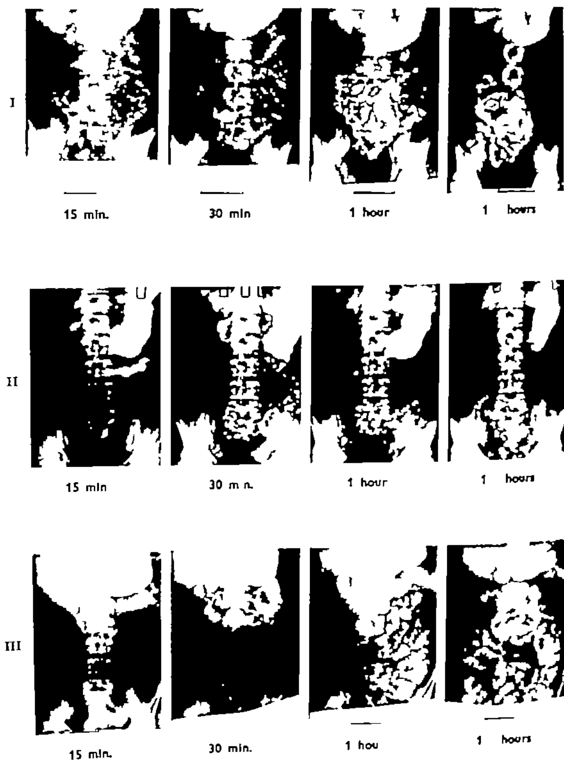


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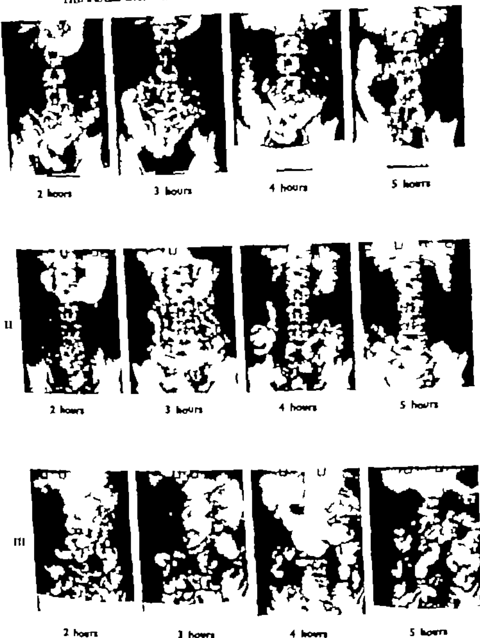


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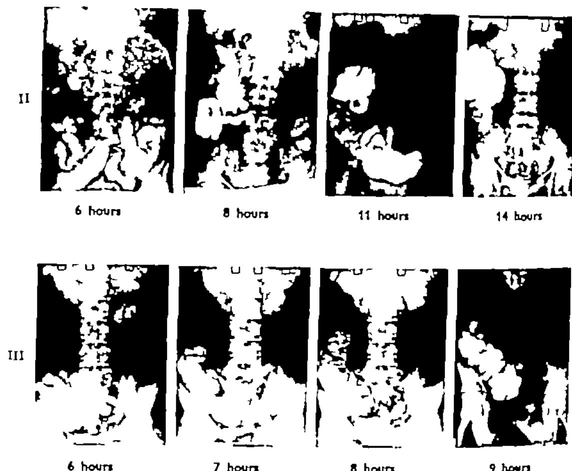


Fig 3 The final films of the pathological series (II and III) taken up t 14 and 9 hours respec tl ely (small intestin transit completed) II Case 1 in tabl IV III Case 5 in tabl III

Table I Composition of the nutritive part of the meal (Caloric content 1 25 cal ml)

	g	Fat	Carbo hy drate	Pro- tein
Corn oil	74	74	—	—
Skim milk powder	126	0.5	50	63
Dextrose	138	—	138	—
Polyethylene glycol	5	—	—	—
Serum albumine	1	—	—	1
Water	1 000			
		74.5	188	64
	mg ml	60	150	50

Table II The small intestine transit time (gi en in hours) in 30 normal cases (From Mattsson, Perman Lagerlöf 1960)

	Stomach completely empty	Contrast reaches S 5 S 1 level	Meal appears i caecum	Meal has left term. ileum
Mean value	4.2		1.9	6 3
Range	2.3–8	0.25–0.50	0.5–4	4–8
95 confidence l mit	2.0–7 1		0.5–3.9	4.2 8.7

Non- bsorbable reference substance

Table III Pancreatic achylia

Case no.	Sex Age	Case history	Secretin test		d-Xylose test g/5 hrs	Vitamin A test I.U./100 ml Peak level
			HCO m mol	Amylase units		
1	M 47	Relapsing pancreatitis. Steatorrhoea since 1956 and weight loss.	0.9	11	—	—
2	M 56	Carcinoma of the head of the pancreas with jaundice and steatorrhoea.	1.1	15	—	—
3	M 52	Chronic relapsing pancreatitis with calcification. Steatorrhoea. Pain. Total pancreatectomy 1958. Diabetes mellitus after operation.	1.0	0.1	7.4	375
4	F 35	Relapsing pancreatitis with multiple stones in the pancreatic ducts. Diabetes mellitus. Steatorrhoea.	0.9	1.4	7.1	385
5	M 42	Carcinoma of the pancreas with metastases. Steatorrhoea. Weight loss.	0.5	17.8	—	—
6	F 78	Steatorrhoea. Weight loss. Anorexia. Anaemia. Cancer pancreas?	0.4	0	—	—
7	M 45	Relapsing pancreatitis with calcifications, steatorrhoea and weight loss.	1.9	13.2	7.8	675
8	M 55	Relapsing pancreatitis with calcification, steatorrhoea and weight loss.	0.2	7.2	—	—
9	M 54	Relapsing pancreatitis and cirrhosis of the liver. Steatorrhoea.	6.1	13.9	8.1	311
10	F 69	Carcinoma pancreatis. Weight loss. Anaemia. Steatorrhoea.	0.5	2.9		318

rather like [illegible] gruel and *palatable* to most patients, was given in [illegible] volume of 300 ml [illegible] 20 C (2 [illegible] volumes barium powder and 3 volumes of the nutrient mixture). The caloric content [illegible] about 225 calories. The patients were if possible up and about [illegible] and had their luncheon four hours after the test meal. The evening meal was eaten as usual.

The films were obtained [illegible] fixed intervals, the first one 15 minutes after ingestion of the meal — usually at 8 [illegible] clock. Subsequent films were taken at 30 min, 1 hour 1 1/2 hours, 2 hours, and thereafter [illegible] intervals of one hour until all the contrast medium had left the ileum. All patients were examined supine in the p.a. projection with the roentgen tube above the patient.

The analyzing of the passage of the test meal was made by recording when the first portion of the meal had reached the L 5—S 1 level (1) when the fundus of the caecum was filled (2) when the stomach was com-

Table IV Non tropical sprue syndrome

Case no.	Sex Age	Case history	Secretin test		d Xylose test g/5 hrs	Vitamin A test I U /100 ml Peak level
			HCO mmol	Amylase units		
1	M 50	Non tropical sprue of 20 years duration. Autopsy 1959 showed multiple reticulum cell sarcomata.	13.7	134	2.2	120
2	F 45	Megaloblastic anaemia and steatorrhoea for one year Remission on folic acid treatment.	12.3	356	3.8	236
3	F 51	Non-tropical sprue since the age of 25 years. Steatorrhoea, weight loss, anaemia.	13.1	557	2.8	325
4	M 17	Non tropical sprue. Remission on gluten free diet and folic acid.	6.5	303	1.2	390
5	F 46	Steatorrhoea, weight loss, anaemia of megaloblastic type.	16.8	278	3.8	263
6	M 50	Steatorrhoea, weight loss, anaemia of 1 year's duration.	21 0	146	2.1	1,269
7	M 44	Steatorrhoea, weight loss, anaemia.	19.7	740	1 6	—

Normal values:
Secretin test: HCO_3- 8.0—25.0 mmol/1 hour
Amylase: 300—1 000 A.U 1 hour
d-Xylose test: > 4 4 g 5 hours
Vitamin A absorption test: > 500 I.U 100 ml within 4—6 hours (peak level)

pletely empty (3) and when all the contrast meal had reached the colon (4)

The small intestine transit time examined radiographically with this method gave moderately uniform results in a series of 30 persons without known disease in the gastro-intestinal tract. The range and means are given in table II (Mattsson Perman & Lagerlöf 1960)

Material

Seventeen cases of steatorrhoea wer ex amined. Ten of the patients with pancreatogenic steatorrhoea are described in table III Three of them, cases no. 2, 5 and 10 were due to carcinoma of the pancreas verified at operation or autopsy The remaining seven patients of this group all had chronic relapsing pancreatitis with l ttle or no response to secretin and with teatorrhoea of varying degree Case 3 was later subjected to a total pancreatectomy partial gastrectomy and duodenectomy for chroni relapsing pancreatitis.

Table IV shows the seven cases of steatorrhoea of other aetiology All had normal pancreatic function assessed from the secretin tests. The d xylose absorption test was pathological n all the cases except one where a borderline value was obtained. The clinical diagnoses were jejuno-ileitis or non-tropical prue

Results

In the patients with pancreatic achylia considerably longer times for transit time were noted than for normals. Thus the

Table I. The small intestine transit time in pancreatic achylia (time given in hours)

Case No.	Sex age	Stomach completely emptied	Contrast reaching L_5–S_1 level	Contrast appearing in caecum	Contrast has left distal ileum
1	M 47	3	0.5	9	16
2	M 58	12	0.5	7	24
3	M 32	4	0.25	4	7
4	F 35	9	0.5	6	9
5	M 42	7	0.5	6	9
6	F 78	4	0.25	7	10
7	M 43	4	0.25	6	8
8	M 33	9	0.25	4	13
9	M 54	12	0.25	3	12
10	F 68	9	0.5	3	9
Mean value		6.9		5.3	11.3
95 % confidence limit*		1.4–16.8		2.3–9.1	3.4–23.8
Range		3–12		3–9	7–24

The square root transformation was performed to normalize the skew distribution

Table VI. The small intestine transit time in non-tropical sprue syndrome (time given in hours)

Case No.	Sex age	Stomach completely emptied	Contrast reaching L_5–S_1 level	Contrast appearing in caecum	Contrast has left distal ileum
1	M 50	9	1	8	14
2	F 45	9	0.25	4	12
3	F 51	7	0.25	4	9.5
4	M 17	10.5	1.5	3	18
5	F 46	7	0.25	6	12
6	M 50	4	0.25	3	12
7	M 44	4	0.50	4	15
Mean value		7.0		4.7	13.1
95 confidence limit*		2.1 14.8		1 9.4	.5–20.3
Range		4–10.5		3–8	9.5–18

The square root transformation was performed to normalize the skew distributions.

Table VII. Statistical differences between the normals and the two pathological groups. Each pathological group was treated separately in comparison with the normal group. A square root transformation was used to normalize the skew distribution

	No. of cases	Stomach completely emptied	Meal appears in caecum	Meal has left terminal ileum
Pancreatic achylia	10	= 3.94 $p < 0.001$	= 8.5 $p < 0.001$	$t = 6.11$ $p < 0.001$
Sprue syndrome	7	= 4.24 $p < 0.001$	$t = 6.55$ $p < 0.001$	= 10.47 $p < 0.001$

time required for the first part of the test meal to reach the caecum was 3 to 9 hours. The whole meal left the small intestine later than in the normals The same prolongation of the small intestine transit time was found in patients with steatorrhoea on the basis of non-tropical sprue syndrome. Compare table V VI and VII of which the last one gives the comparison with the normals

Both in the pancreatic achylia group and in that of sprue longer times were found than in the normal material. On the contrary no significant difference exists when comparing the passage of the test meal in the patients with sprue and in those with pancreatic achylia

Typical series of films demonstrating the difference between normals and the two steatorrhoea groups — pancreatogenic achylia and sprue — are shown in figs. 1—3 The two pathological cases correspond to the patient no 5 in table III and the patient no 1 in table IV Fig 1 shows the films taken up to 1 1/2 hours after the ingestion of the meal fig 2 those taken after 2 up to 5 hours and fig 3 the following In the beginning the considerable difference in the degree of filling of the small intestine between normal and pathological cases is striking The prolongation of the transit time for the pathological groups is considerable and the small intestine not emptied until after 9 and 14 hours respectively (fig 3)

Discussion

Knowledge has been limited concerning the time required for a meal to pass through the small intestine. The small intestine transit time examined with the routine barium-water mixture is not comparable with the rate of passage of food The special food barium meal used here, which is kept constant regarding its composition, volume and temperature, seems to give a more adequate result as it is a meal 'labelled with roentgen contrast. The diverging results obtained by earlier authors might be explained by the lack of physiological stimulation of the food.

In the present material the transit times of ordinary barium water mixtures without food were remarkably different from that of the food barium meal in all cases where both methods have been applied. For instance in case no. 1 (table III) the food-barium meal reached the caecum after 9 hours, but the barium-water mixture after only one hour The barium water mixture always passes more rapidly This observation explains the divergent results of earlier authors.

The difference in transit time conditions between the cases of steatorrhoea and the normals as demonstrated with follow up examination with the barium-food meal is evident The distribution of the contrast media in the small intestine at different points of time seems to be so characteristic for the cases of steatorrhoea that an important value might be attached to the roentgen examination in the diagnosis of the syndrome.

Summary

The small intestine transit time has been studied with a special food-barium meal in 17 patients with steatorrhoea, 10 with pancreatic achylia and 7 with the sprue syndrome.

The transit times of these two groups in comparison to those of normals are significantly longer Between the two pathological groups however no statistical difference has been observed in this material

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Acta Medica Scandinavica. Vol. 171 fasc. 3, 1962

From Medical Department B, Aker hospital, Oslo, Norway

Combined Treatment of Thyrotoxicosis with Perchlorate and Propylthiouracil

By

Øistein Blobstad and J. H. Vogt

A thyrotoxic patient may be made euthyroid by surgery or treatment with radioactive iodine, or by thyrostatic drugs. The latter method has the advantage that treatment can be stopped whenever desired, or adjusted to the need of the patient in the course of treatment.

Thiocyanate and other monovalent anions, of which perchlorate has been shown to be most useful (3 5 8, 10 14) inhibit the trapping of iodine in the thyroid gland (3 11 12) The inhibition may be overcome by ingestion of large amounts of iodine (9 10 12) Concomitant use of iodide results in little or no therapeutic effect (1 9) The effect of perchlorate is reduced when the gland is saturated with iodine, but the amount of iodine trapped may be reduced by perchlorate under certain circumstances (16).

A dosage of 1,000 mg of perchlorate a day has about the same therapeutic effect as methyl- (and propyl-) thiouracil 600 mg daily for two weeks, followed by 300 mg daily Six hundred mg of perchlorate gives about the same result as 60 mg carbimazol for two weeks followed by 30 mg daily (5 14)

Thiocarbamides and other similar drugs inhibit the biosynthesis of hormonally active iodothyronines from iodine and thyrosine (7 11) Methylthiouracil, propylthiouracil and carbimazol are actually the most frequently used. Increasing doses of thiocarbamides give increasing frequency of toxic reactions (5, 14) propylthiouracil being the least toxic (2, 15)

Concomitant ingestion of iodide does not prevent the therapeutic effect of the thiocarbamides.

Both perchlorate and the thiocarbamides may give hypertrophy of the gland.

Combined treatment of thyrotoxicosis with perchlorate and propylthiouracil seems rational because their points of attack are different. It may be anticipated that relatively small doses of each drug can be given thus hoping for a reduction in the frequency of toxic reactions. Iodide may be given without delay should such medication be judged

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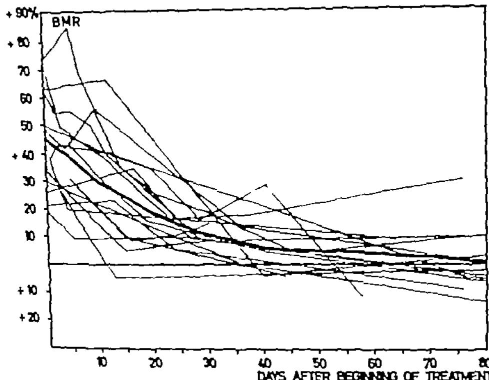


Fig. 2. Basal metabolic rate during combined treatment with $KClO_4$ and propylthiouracil. The accentuated line gives the average course.

plasma and the basal metabolic rate. In thirteen cases examinations with ^{131}I and determinations of proteinbound iodine were done; two further patients had examinations with ^{131}I only and four with PBI only. In four cases these examinations seemed quite superfluous, the diagnosis being beyond doubt.

Nine patients received an initial dosage of 600 mg of potassium perchlorate and 400 mg of propylthiouracil daily; twelve patients had 400 mg of perchlorate and 300 mg of propylthiouracil, two patients 400 mg of each drug. After an average of 6 weeks the dose was reduced by about 30 % for both drugs, and further reduction was effected as soon as permitted by the condition of the patient.

The efficiency of the treatment was judged by serial determinations of the cholesterol content of the blood plasma, the basal metabolic rate, body weight, in some cases by the serum proteinbound iodine, and by clinical evaluation by one of us (J. H. V.).

Results

During the first two weeks of treatment a distinct improvement in the patients' condition was usually observed. In nineteen cases who received the combined treatment without any interruption, the patients were judged as being

Table I

Uncomplicated thyrotoxicosis	15
Auricular fibrillation	3
Angina pectoris	1
Exophthalmos	2
Sequela of pulm. tub.	1
Pulmon. emphysema	1
Disseminated sclerosis	1
	Total 23

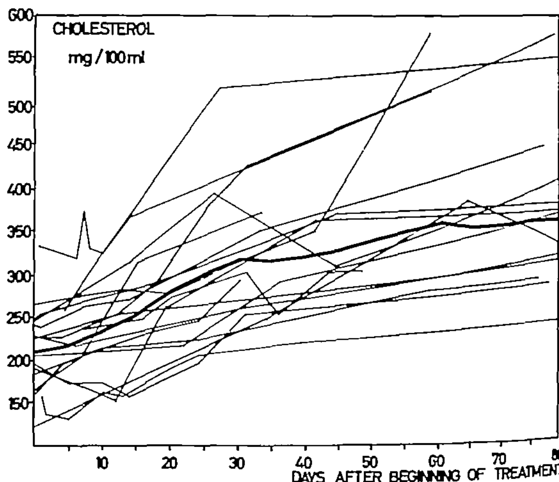


Fig 1 Cholesterol in plasma during combined treatment with KClO and propylthiouracil. The accentuated line gives the verage course.

desirable in cases where in the course of treatment surgical intervention should be considered the best choice. As an alternative thyroxine or triiodothyronine may be given as preoperative treatment, without stopping the administration of thyrostatic drugs (4 6)

None of the drugs will interfere with subsequent treatment with radioactive iodine, should such treatment be decided upon. The great advantage of flexibility and freedom of choice will thus be preserved.

Fewer cases of resistance to drug treatment may be expected, such cases being mostly due to unknown or forgotten ingestion of iodide or iodine.

No such series of combined treatment with an anion and a thiocarbamide has been previously published.

Material

Since June 1960 we have treated 23 cases of thyrotoxicosis with a combination of potassium perchlorate and propylthiouracil, 4 men and 19 women Their ages ranged from 16 to 68 years, a erage 50 years. The duration of the disease judged by the information given by the patients, varied from 1 to 48 months, average 11 months. Fifteen of the 23 patients had an uncomplicated thyrotoxicosis. The secondary diagnosis in the other cases is given in table I

The diagnosis was based on anamnestic information clinical observation serial determinations of cholesterol content in blood

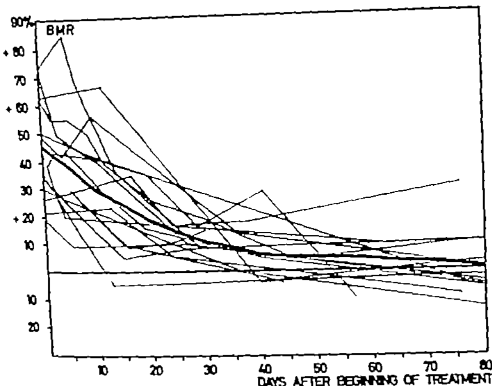


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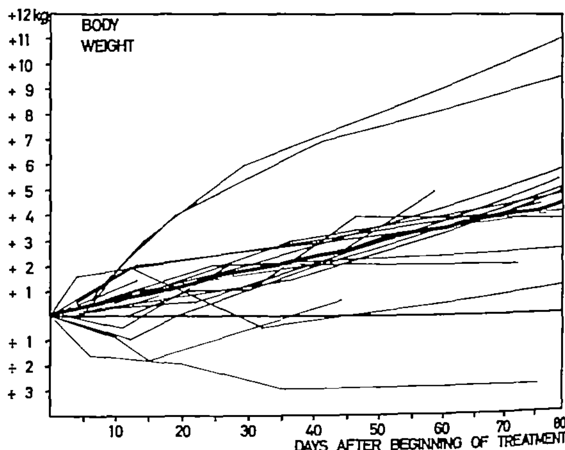


Fig 3 Changes in body weight during combined treatment w th KClO and propylthiouracil The accentuated lin gives th a erage course.

euthyroid after 6 to 11 weeks, average 7 5 weeks.

No certain difference in response was found in the cases receiving the highest or the lowest dosage levels.

Plasma cholesterol content was noted to increase after 1 5 to 2 weeks of treat ment (fig 1)

In the cases where treatment was stopped a reduction of the cholesterol content was found after 5 to 7 days.

A distinct fall in the basal metabolic rate was noted after 1 to 2 weeks of treat ment (fig 2) The average was ÷ 15 after 25 days of treatment

The body weight increased but after a somewhat variable period In all cases but four a distinct increase in body weight was noted after two weeks of treat ment (fig 3)

Twenty-one patients had an enlarged thyroid gland before treatment In one of them the size of the gland augmented. This patient eventually was treated with radioactive iodine. In seventeen of them the size of the gland did not change and in three of them it decreased

In two patients with severe exophthalmos X ray treatment of the hypophysis was given in addition to combined drug treatment. The exophthalmos did not progress and both of them responded favourably to treatment.

The result of treatment was judged as good when the patient was found to be clinically euthyroid, with a normal

basal metabolic rate, a distinct increase in serum cholesterol level and of body weight. In nineteen patients this was the case.

In one case with heart failure and pulmonary fibrosis there was but little improvement after five weeks, whereafter she was given treatment with radioactive iodine.

In another case there was but slight improvement after 20 weeks of combined treatment. This patient had a nodular goitre. Serum cholesterol increased from 203 to 323 mg and the body weight increased by 1 kg the basal metabolic rate however did not change and she was still judged thyrotoxic. Possibly the dosage was too low in this case (400 mg of perchlorate and 300 mg of propylthiouracil) as nodular goitre frequently necessitates a higher dosage (2)

Side-effects

One patient developed agranulocytosis due to propylthiouracil after two weeks of treatment. In one patient perchlorate had to be stopped because of nausea, and in a third patient propylthiouracil was stopped because of a rash which did not prevent continuation of perchlorat (table II)

Discussion

The results we have obtained may be compared with the results of Crooks and Wayne (5) where euthyroidism was obtained on the average after 13 1 weeks with perchlorate 600 mg daily after 9 1 weeks with methylthiouracil 600 mg daily after 12.3 weeks with carbimazol 60 mg daily and after 9 4 weeks with perchlorate 1,000 mg daily The mode of evaluation was more elaborate in the cases of Crooks and Wayne, and naturally a comparison may be fallacious.

Table II

Good effect	19
No effect	2
Side-effects	
Agranulocytosis	1
Nausea	1
Exanthema	1
Augmented goitre	1
Discontinued	2

Recently a warning has been issued against the use of potassium perchlorate, based on the publication of 5 cases of agranulocytosis or neutropenia, and 5 cases of aplastic anaemia (17) In the latter cases the initial dosage was 1 000 600 and 800 mg the total dosage 108, 102 and 160 g It is possible that reduction of the initial dosage to 500 or 400 mg will prove necessary the concomitant use of propylthiouracil permitting a further reduction after an average of 6 weeks.

Summary

Of twenty-three patients with thyrotoxicosis treated with a combination of perchlorate and propylthiouracil the initial effect was judged as good in nineteen cases. They were found to be euthyroid after an average of 7.5 weeks.

One patient developed agranulocytosis due to propylthiouracil, in one patient perchlorate had to be topped because of nausea, a third patient developed an exanthema due to propylthiouracil. In two cases treatment was stopped because of poor effect after 5 and 20 weeks.

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Thyroid Auto-Antibodies

A Study on the Occurrence of Circulating Thyroglobulin Antibody and Complement fixing Thyroid Auto-Antibody and an Evaluation of the Importance of these Antibodies in the Development of Post-Operative Myxoedema

By

TAGE HJORT and ERIK F MOGENSEN

The discovery made by Roitt et al. in 1956 (32) that the increased amounts of gamma globulin in the serum of patients with Hashimoto's disease was referable to the occurrence of thyroid auto-antibodies marked the starting point for intensive research. It was soon demonstrated that the original precipitating antibody was thyroglobulin antibody (Roitt, Campbell and Doniach 1958 (29) Witebsky et al. 1958 (37)) At about the same time American research workers (Witebsky and Rose 1956 36) Rose and Witebsky 1956 (33)) observed that rabbits after intracutaneous injection of autologous thyroid extract to which Freund adjuvant had been added responded with the formation of thyroglobulin antibody and simultaneously the remaining thyroid tissue in the rabbits showed histological changes of exactly the same character as those seen in the thyroid gland of patients with Hashimoto disease. It was therefore reasonable to assume that the formation of a thyroglobulin antibody — excited by leakage of thyroglobulin from the thyroid gland — was the direct cause of Hashimoto's disease (Doniach and Roitt 1957 (9)) After it has been disclosed that several different thyroid auto-antibodies exist and that the presence of auto-antibody need not necessarily lead to the development of Hashimoto disease, our knowledge of the pathogenetic significance of the thyroid auto-antibodies is very uncertain.

In addition to thyroglobulin antibody which can be demonstrated by precipitation or by Boyden's haemagglutination technique, there exists a complement fixing thyroid auto-antibody

This antibody can be demonstrated by complement-fixation reaction, in which

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findings and the clinical condition after thyroidectomy we have made an attempt to evaluate the clinical significance of the antibodies. As the incidence of thyroid disease varies widely in different parts of the world, we have also given a brief survey of the frequency of thyroid auto-antibodies in patients with thyroid disorders in Denmark, since this has not previously been studied. (Only one Danish study on thyroid antibodies has been published. (Mogensen 1958) (25).)

Methods

Thyroglobulin antibody was demonstrated by Boyden haemagglutination technique which was performed essentially as described by Roitt and Doniach (1958) (30). Boyden reaction (Boyden, 1951 (3)) is based on the phenomenon that proteins (antigens) can be bound to the surface of red blood cells which have previously been subjected to gentle tannin treatment, the blood cells are coated with the antigen. If these coated blood cells are added to a serum dilution containing the corresponding antibody in a complete form, the antibody will cause agglutination of the blood cells.

Sheep erythrocytes were used. After washing, tannin treatment and coating with a purified thyroglobulin fraction (made from thyroid tissue by the method of Derrien et al. (1948) (6)) the erythrocytes were treated with formalin as described by Fulthorpe (1957) (12). The formalin-treated erythrocytes had shelf life of at least 6 months.

After inactivation (at 56° C for 30 min.) and absorption with formalin-treated erythrocytes which had only been subjected to tannin treatment, the serum was titrated in perspex tray with 1 ml cups in the dilutions 1:5, 1:25, 1:125, ... 1:5 To 0.1 ml serum dilution was then added 0.1 ml of a 1 % suspension of formalin-treated, thyroglobulin-coated erythrocytes. After standing overnight at room temperature the reading was performed. The highest serum concentration (1:5) to which tannin-treated erythrocytes were added was used as control.

The semi-micro method described by Donnelly (1951) (10) was used for the demonstration of complement-fixing antibody. The test procedure was exactly as reported by Roitt and Doniach (1958) (30). Thus 2 minimum haemolytic doses of complement were used, titrated in the presence of the antigen. Fresh extracts from thyrotoxic glands were used as antigen. The optimum antigen concentration was determined by titration with known positive sera, and the anticomplementarity of the antigen was checked. In order to ensure that the antibodies were strictly organ-specific, complement-fixation experiments with fresh liver extract as antigen were performed simultaneously.

Clinical material

For a period of more than 2 years (1958—1960) all patients with thyroid disease admitted to the Medical University Clinics of Aarhus Kommunehospital were studied for thyroglobulin antibody and complement fixing antibody. At the same time number of blood donors without thyroid disorders were studied in order to disclose the frequency of thyroid auto-antibodies in normal subjects.

In addition, as far as possible all patients who underwent partial thyroidectomy in the department of surgery L. Aarhus Kommune hospital, during certain period in 1958—1959 were subjected to antibody determinations before and after operation. At several check-up examinations during the first few months after operation the immunological investigations were repeated, and determinations of the basic metabolic rate and protein-bound iodine were simultaneously performed. In the patients who presented symptoms of incipient myxoedema, regular examinations were continued. At least 12 months after the operation (or in four cases, before that time) questionnaire was submitted to the patients in whom the check-up examinations had been discontinued. If the answers received aroused the slightest suspicion of impaired thyroid function, the patients concerned were requested to appear for closer examination. A few patients with whom contact had been lost are not included in the analyses.

it reacts with an intracellular antigen which occurs in the greatest amounts in thyroid tissue from thyrotoxic patients (Trotter et al 1957 (34) Belyavin and Trotter 1959 (1)) The cytoplasmic localization of the "complement fixing" auto-antigen has been confirmed by Coons fluorescent antibody technique (Holborow et al. 1959 (18)) Both auto-antibodies were first found in patients with Hashimoto s disease, but it has since appeared that they also frequently occur in other thyroid disorders. In their latest paper Roitt and Doniach (1960 (31)) reported that at least one of these auto-antibodies is present in the serum in 97 % of patients with Hashimoto s disease. In "primary myxoedema they found auto-antibody in 83 % in thyrotoxicosis in 63 % and in non toxic goitre in 33 % of the cases. In an Australian series Hackett et al. (1960) (15) observed thyroglobulin antibody in 9 % of 102 normal subjects (blood donors) and in 18 % of 387 medical patients without thyroid disease. The results of MacKay and Perry (1960) (22) are on the same level.

Neither thyroglobulin antibody nor complement fixing antibody has any cytotoxic effect on thyroid cells in tissue culture. However in some patients with Hashimoto s disease, Pulvertaft et al (1959) (28) demonstrated a third thyroid auto-antibody the "cytotoxic factor which according to subsequent investigations (Irvine 1960) (19) also seems to occur frequently in thyrotoxicosis and myxoedema.

After intracutaneous injection of a saline extract of human thyroid tissue Buchanan et al. (1958) (5) observed a positive skin test in some patients with Hashimoto s disease and primary myxoedema. Thus there may be an immune reaction of the delayed type of hypersensitivity — a reaction which is usually referred to cells (lymphocytes) and not to circulating antibodies. This reaction has gained particular interest since Miescher et al. (1961) (24) in guinea-pigs have demonstrated a correlation between the occurrence of experimental thyroiditis and the presence of positive skin tests.

While several studies on the frequency with which the auto-antibodies occur in various thyroid disorders are available few investigations have been performed in which the immunological findings have been related to the course of the disease and the treatment given. The reports published by Doniach and Roitt (1957) (9) and Doniach, Hudson and Roitt (1960) (8) show that the conditions in which thyroid auto-antibodies are present in the serum in a high titre — cases of auto-immune thyroiditis — often lead to hypothyroidism, the final stage of the auto-immunization being either "primary myxoedema or Hashimoto s disease. Both during treatment with desiccated thyroid and after partial thyroidectomy a fall in the amounts of circulating auto-antibody usually occurs, but nevertheless cases are on record in which thyroidectomy was followed by the occurrence of thyroglobulin antibody (Paine et al 1957) (27) Blagg (1960) (2) studied the occurrence of thyroglobulin antibody in patients with thyrotoxicosis before and after treatment with radio-iodine (^{131}I) As it appeared that, after treatment, thyroglobulin antibody was found with a higher frequency in the patients who had become hypothyroid Blagg expressed the view that an immunisation may play a part in the development of hypothyroidism

The purpose of the present study was to throw light on the influence of thyroidectomy on the auto-immune processes. By a comparison of the immunological

concerned both antibodies were present in relatively high titres (The titre in the haemagglutination test was 3125 In the complement fixation-test 64)

In the analysis of the occurrence of thyroid auto-antibody in patients with myxoedema, we distinguished between untreated cases and cases which had been under treatment with desiccated thyroid for 12 months or more. Antibodies were revealed in nine out of ten patients in the former group as against only in two out of six in the latter One of the last two had co-existing macroglobulinaemia of the Waldenström type and diabetes mellitus. The complement-fixing antibody in this patient was not thyroid-specific, but reacted equally (titre 1024) with all the tissue extracts (thyroid, liver kidney and parotid) against which it was tested. This case is thus similar to those of hepatic disorders, lupus erythematosus and macroglobulinaemia, in which Gajdusek (1958) (13) and MacKay and Gajdusek (1958) (21) found antibodies which by com plement-fixation test reacted with both homologous and heterologous tissue extracts. Accordingly while it can justifiably be presumed that the antibody production in this patient was not excited by the thyroid disease it cannot be ruled out that her myxoedema (and diabetes mellitus?) may be related to the production of the abnormal antibodies.

Although we studied only a small number of patients with myxoedema, the difference in the frequency with which the thyroid auto-antibodies occurred in the untreated and treated groups of patients nevertheless gives the impression that the circulating auto-antibodies may disappear under treatment with desiccated thyroid.

At least one of the two thyroid auto-antibodies was demonstrated in 35 (or 55 %) of the 65 patients with thyrotoxicosis studied This is also in agreement with the findings of Roitt and Doniach (1960) (31) who reported that antibodies were present in 63 %. It must be emphasised that at the present time it is an unsettled question if thyroglobulin antibody and complement fixing antibody are of the same pathogenic significance. The reason why we have calculated the frequency of patients with at least one of the antibodies is that this facilitates comparison with the results obtained by other investigators.

Even though great importance cannot be attached to the titres as such It was seen that the values obtained were on the whole lower in the thyrotoxicosis group than in the patients with myxoedema Thus in the hyperthyroid patients the highest titre-value observed in the tanned cell-test was 3 125 while 4 of the patients with untreated myxoedema showed higher values. The highest titre observed in this group was 2,000,000 In an attempt to divide the patients with thyrotoxicosis into untreated and treated cases it was not possible to demonstrate any definite difference in the frequency of antibodies in the two subgroups. The patients had received various forms of treatment Partial thyroidectomy administration of Neo-Mercazole (2-carbethoxy-thiamazol) methyl thiouracil or — in two cases — [131]I Only patients who underwent operation were controlled in such a way that is was possible to assess the influence of the treatment on the thyroid auto-antibodies. However it is noteworthy that the two [131]I-treated patients, who both had complement fixing antibody in the serum before the treatment, revealed a distinct increase in the amounts of antibody after treatment (cf. Blagg 1960) (2)

Thyroglobulin antibody or comple-

Table I The occurrence of thyroglobulin antibody (TGA) and complement-fixing antibody (CFA) in 132 normal subjects (blood donors) and 160 patients with thyroid disorders

Diagnosis	No. of cases tested	Presence of both TGA and CFA	Presence of TGA alone	Presence of CFA alone	No anti bodies present	At least one antibody present (%)
Normal subjects	132	0	4	1	127	4
Hashimoto disease	1	1	0	0	0	–
Myxoedema, untreated cases	10	5	3	1	1	90
Myxoedema, treated cases	6	0	1	1	4	33
Thyrotoxicosis	65	10	11	15	29	55
Non toxic goitre / Non-toxic adenoma	78	0	2	3	73	6

Results

A Occurrence of thyroid antibodies in the clinical material as a whole

The frequency of thyroglobulin anti body and complement fixing antibody in the normal subjects and patients is shown in table I

Thyroid auto-antibody was detected in only five of the normal subjects (4 %) Small amounts of thyroglobulin antibody were demonstrated in four men (at the haemagglutination reaction the titre was 5 in two 25 in one, and 125 in one case) and complement fixing antibody in one man (titre 4) These donors were ex amined again 6 months later Thyroglobulin antibody was still present in the first four while complement fixing anti body could no longer be found in the fifth subject. In the evaluation of this series of normal subjects, it must be con sidered that there was a strong male pre ponderance (97 men as against only 35 women) and that the majority of the subjects belonged to the younger age groups (77 under 40 55 over 40 years) Both these shifts in the normal distri bution must be expected to give a tend ency to a low frequency of antibodies since these are reported to be most frequent in elderly women. However the frequency observed among our normal subjects shows fairly good agreement with the figures reported by British investigators. Thus, Roitt and Doniach (1958) (30) Owen and Smart (1958) (26) and Hill (1961) (16) found thyroglobulin anti body in 5—6 % of the individuals in their control series. These investigators, however did not study healthy subjects, but a group of patients without thyroid disease According to the reports of Hackett et al (15) (see above) and MacKay and Perry (22) thyroglobulin antibody may be expected to occur with a somewhat higher frequency in such series than in healthy subjects.

The rare occurrence of thyroid auto-antibodies, in particular of complement fixing antibody in persons without clinical signs of thyroid disease is obviously decisive for the importance which can be attached to the demonstration of these antibodies in patients with thyroid dis-orders.

Hashimoto s disease is a rare disease in Denmark. This diagnosis was made only in one among the 160 patients with thyroid disease studied In the patient

concerned, both antibodies were present in relatively high titres. (The titre in the haemagglutination test was 3125, in the complement fixation-test 64.)

In the analysis of the occurrence of thyroid auto-antibody in patients with myxoedema, we distinguished between untreated cases and cases which had been under treatment with desiccated thyroid for 12 months or more. Antibodies were revealed in nine out of ten patients in the former group as against only in two out of six in the latter. One of the last two had co-existing macroglobulinaemia of the Waldenström type and diabetes mellitus. The complement-fixing antibody in this patient was not thyroid-specific, but reacted equally (titre 1024) with all the tissue extracts (thyroid, liver, kidney and parotid) against which it was tested. This case is thus similar to those of hepatic disorders, lupus erythematosus and macroglobulinaemia, in which Gajdusek (1958) (15) and MacKay and Gajdusek (1958) (21) found antibodies which by a complement-fixation test reacted with both homologous and heterologous tissue extracts. Accordingly, while it can justifiably be presumed that the antibody production in this patient was not excited by the thyroid disease, it cannot be ruled out that her myxoedema (and diabetes mellitus?) may be related to the production of the *abnormal antibodies*.

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Thyroglobulin antibody or comple

Table II The occurrence of postoperative myxoedema among 39 patients subjected to partial thyroidectomy related to the antibody findings before operation

Antibody findings before operation	Hyperthyroid before operation		Euthyroid before operation	
	Total no.	No. of cases with postop. myxoedema	Total no	No. of cases with postop myxoedema
Presence of both TGA and CFA	3	1	0	0
Presence of TGA alone	2	0	1	0
Presence of CFA alone	3	3	1	(1)
No antibodies present	8	0	21	0
Total	16	4	23	¹(1)

TGA = thyroglobulin antibody
CFA = complement-fixing antibody

¹ Transient hypothyroidism observed.

ment fixing antibody was demonstrated in only 6 % of the 78 patients with non toxic goitre or adenoma In one case complement fixing antibody was present in a moderately high titre (16) This patient a woman aged 56 had 35 years previously been treated with roentgen irradiation for hyperthyroidism Apart from this case, the antibody findings in the euthyroid patients were similar to those in the normal subjects In this group our results differed widely from those reported by Roitt and Doniach (1960) (31) who found antibody in 33 of the patients with non toxic goitre In Australia Hackett et al. (1960) (15) revealed thyroglobulin antibody in as many as 10 out of 16 euthyroid patients with goitre (i e 65 %)

B The occurrence of thyroid auto-antibodies in thyroidectomised patients in relation to the development of postoperative myxoedema

Check-up examinations were performed on 16 hyperthyroid and 23 euthyroid patients after partial thyroidectomy The pre-operative antibody findings in these 39 patients are shown in table II Whereas eight of the 16 hyperthyroid patients had auto-antibody in the serum this was the case in only two of the 23 euthyroid patients In the table the four cases of post operative myxoedema which occurred are also related to the pre-operative findings. It is seen that these four patients had all had complement fixing antibody in the serum before operation On the other hand postoperative myxoedema did not develop within the first 12 months in any of the patients in whom no antibody at all, or only thyroglobulin antibody had been detected before operation. Although the figures are too small for definite conclusions, the results nevertheless suggest that there is a difference in the pathogenic significance of the two antibodies.

Table III shows the antibody titres before operation and 6 months after operation in the 10 patients in whom auto-antibody was revealed in the serum pre-operatively The clinical condition before and after operation is also stated It is seen that the amount of complement fixing antibody decreased after operation in most of the patients, including those in whom postoperative myxoedema developed As regards thyroglobulin antibody both higher and lower titres were obtained after operation and in one of the patients (case 3 table III) thyroglobulin antibody was present 6 months after operation i e at a time when myxoedema had developed

Table III Antibody titres before and about 6 months after partial thyroidectomy in 10 patients (all women) in whom antibody was present before operation

Case no.	Age (years)	Status before operation	Thyroglobulin antibody		Complement-fixing antibody		Status after operation
			Before op.	After op.	Before op.	After op.	
1	14	Hyperthyroid	125	625	8	0	Euthyroid
2	15	Hyperthyroid	125	125	16	16	Euthyroid
3	24	Hyperthyroid	0	25	8	8	Postop. myxoedema after 4 months
4	30	Hyperthyroid	125	25	0	0	Euthyroid
5	33	Hyperthyroid	0	0	4	< 4	Postop. myxoedema after 7 months
6	45	Hyperthyroid	125	25	0	0	Euthyroid
7	56	Hyperthyroid	0	0	8	< 4	Postop. myxoedema after 6 months
8	70	Hyperthyroid	5	5	16	< 4	Postop. myxoedema after 9 months
9	56	Euthyroid	0	0	16	8	Transient hypothyroid
10	61	Euthyroid	5	0	0	0	Euthyroid

Special interest attaches to the three patients (cases 1, 2 and 9) in whom postoperative myxoedema did not develop although complement fixing antibody was present in the serum. It is noteworthy that two of these patients were very young (14 and 15 years). The third patient — the aforementioned 56-year-old woman, who had been hyperthyroid 35 years previously — did not present any clinical signs of myxoedema, but during the first few months after operation the values for protein-bound iodine varied between 2.4 and 3.0 µg% and the basic metabolic rate ranged from −14 to −12. Thus, during the postoperative months the patient seems to have been hypothyroid.

Two patients in whom no auto-antibodies could be demonstrated before operation revealed small amounts of complement-fixing antibody after operation. However this antibody disappeared again within a couple of months, and the patients did not present signs of hypothyroidism.

Discussion

By the technique used in the present study thyroglobulin antibody and complement fixing antibody were frequently demonstrated in patients with myxoedema or thyrotoxicosis whereas they were rarely observed in euthyroid patients.

The results of tanned-cell-test for demonstration of thyroglobulin antibody vary to some extent from laboratory to laboratory. We have, for example, not been able to find thyroglobulin antibody quite as often as Roitt and Doniach (1960) (31) and not by far so frequently as Hackett et al. (1960) (15) and our titre values have even not been so high. Our results are in better agreement with those obtained by American investigators (Paine et al. 1957 (27), Fahey and Goodman, 1960 (11)). These differences may obviously be referable to geographical variations, but may also to some extent be sought in the test techniques. Boyden's haemagglutination reaction may present

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postoperative myxoedema often developed in patients with this histological picture (Whitesell and Black 1949 (35) Green 1950 (14) Levitt 1951 (20)) which was explained by replacement of the thyroid tissue by the lymphoid infiltrations. However in the light of our knowledge it may be asked to what extent postoperative myxoedema is due to replacement of the active tissue, and to what extent it is referable to an inhibitory influence of the antibodies on thyroid function. A study of thyroidectomised patients in an early stage of the auto-immune process in which the lymphocytic infiltration is still relatively slight would be suitable in throwing light on the importance of the antibodies. As the reserve capacity of the thyroid gland is removed by thyroidectomy it is obvious that the inhibitory influence, if any of the antibodies on thyroid function will manifest itself most distinctly after this operation. Only in one (case 8, table III) of the patients with thyroid auto-antibodies in the serum was the lymphocytic infiltration at the time of operation so pronounced that a significant part of the thyroid tissue had been replaced. In the remaining patients the infiltration was confined to the connective-tissue septa, and in one (case 5 table III) the tissue sections examined failed to reveal any clusters of lymphocytes. As none of the patients showed a significant and persistent increase in the amounts of circulating antibody after operation, it does not seem likely that the lymphocyte infiltration can have increased appreciably postoperatively. The relationship between the presence of complement-fixing antibody before operation and the development of postoperative myxoedema can therefore be taken as evidence in support of the assumption that the complement-fixing antibody exerts an inhibitory action on the function of the thyroid gland

Only small amounts of complement fixing antibody were demonstrated in the patients who became hypothyroid. However the amount of circulating antibody as such is without interest — the decisive factor is the amount of antibody bound in the thyroid gland and so far we have no measure for the latter There need not necessarily be any direct proportionality between the amount of circulating (i. e measured) antibody and the amount which is bound. On the basis of theoretical considerations it might perhaps rather be so that these two factors were inversely related.

The cytotoxic factor was not determined in the present study The occurrence and importance of this factor is not yet fully clarified but Irvine (1960) (19) reported that the occurrence of the cytotoxic factor was always associated with the presence of complement-fixing antibody whereas the reverse was not invariably the case. Accordingly the possibility exists that the patients in whom postoperative myxoedema developed may also have had the cytotoxic factor in the serum, and that it is this factor which has been of significance.

Continued studies are necessary to show how often myxoedema develops after partial thyroidectomy in patients with thyroid auto-antibodies If such studies confirm the existence of a relationship between the occurrence of complement fixing antibody or the cytotoxic factor and postoperative myxoedema, it might be possible to avoid some cases of this condition by a less extensive resection of the thyroid tissue in patients with these antibodies in the serum.

difficulties, since the coated blood cells often show a tendency to spontaneous agglutination Such blood cells will give high titres in positive tests whereas the negative reactions may appear somewhat ambiguous. Only by using perfectly fresh sheep erythrocytes and by treating them with formalin after the coating was it possible to eliminate the tendency to spontaneous agglutination In this way blood cell suspensions with constant reactions are obtained and a number of doubtful reactions are avoided but this is obtained only at the expense of loss in sensitivity

While a gradual disappearance of thyroid auto-antibodies is the commonest consequence of thyroidectomy Pain et al (1957) (27) observed as already mentioned cases in which an auto-immune reaction developed after partial thyroidectomy We have not seen cases in which a strong persistent, auto-immune reaction occurred Only in two patients were small amounts of complement fixing antibody present for short periods, and in one patient who had complement fixing antibody before operation were small amounts of thyroglobulin antibody demonstrated 6 months after operation. However in the assessment of the finding reported by Paine et al. it must be considered at what time the first sample of serum *i e* the one without thyroglobulin antibody was taken. It was reported that some of the samples were taken following operation which involves a serious risk of error since the thyroglobulin antibody titre will often show a considerable fall in the immediate postoperative period because the antibody present may be bound by thyroglobulin which has leaked out (Hjort 1961) (17)

The term "auto-immune thyroiditis is now used in the world literature This disease does not represent a clinically well-defined entity and a definite diagnosis can only be made by the demonstration of thyroid auto-antibodies (either both or one of them) in a high titre in the serum. Severe cases of auto-immune thyroiditis are apparently rare in this country But as these patients sometimes require special treatment, it is of great importance to realise the proper nature of the disease in the individual cases.

"Auto-immune thyroiditis may manifest itself as a subacute condition with general malaise, slight fever swelling tenderness and pain in the neck, *i e* as a true thyroiditis, but it may also be of insidious onset extending over several years In the early phases of the disease the patients may be thyrotoxic, and thyroidectomy may at this stage lead to postoperative myxoedema (Buchanan et al *1961* (4)) These patients may also exhibit clinical symptoms of mild thyrotoxicosis with a firm diffuse goitre and possibly increased ^{131}I uptake in the thyroid gland (because partial destruction of the gland will result in compensatory hyperactivity of the still functioning parts) while basal metabolism is normal or even decreased. In such cases, treatment with desiccated thyroid will usually lead to improvement in the general condition and disappearance of the goitre (McConahey et al. 1959 (23)) with a simultaneous decrease or disappearance of circulating antibody In severe cases, the final stage will be typical myxoedema or in rare instances, Hashimoto s disease (Doniach et al. 1957 1960 (7 8))

The histological appearance of the thyroid gland in patients with auto-immune thyroiditis is marked by diffuse or focal infiltration with lymphocytes and plasma cells associated with a varying degree of replacement of the functioning thyroid tissue It is a well known fact that

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Summary

The occurrence of thyroglobulin antibody (demonstrated by Boyden's haemagglutination reaction) and complement fixing thyroid auto-antibody was studied in a number of normal subjects (blood donors) and patients with thyroid disorders.

By the technique used these auto-antibodies could only rarely be demonstrated in normal persons. Among 132 normal blood donors thyroglobulin antibody was detected in four and complement fixing antibody in one.

The auto-antibodies were revealed with the highest frequency in patients with untreated myxoedema. In 10 patients studied nine had at least one of the antibodies in the serum. Among 65 patients with thyrotoxicosis, auto-antibody was demonstrated in 55 %. On the other hand the findings in 78 patients with non toxic goitre or non toxic adenoma did not differ essentially from those in the normal subjects.

Postoperative check up examinations were performed in 39 patients who underwent partial thyroidectomy and the post operative condition was related to the preoperative antibody findings. Among 16 thyrotoxic patients six had complement fixing auto-antibody in the serum before operation and in four of these six post-operative myxoedema developed. Among 23 non-toxic patients complement fixing antibody was present only in one prior to operation. Transient hypothyroidism was observed in this patient after operation. Postoperative myxoedema did not occur in any of the patients in whom no auto-antibodies, or only thyroglobulin antibody could be demonstrated in the serum before operation.

This relationship between the occurrence of complement fixing auto-antibody before operation and the development of postoperative myxoedema does not necessarily mean that the complement fixing antibody is the cause of postoperative myxoedema, but if the relationship can be confirmed in further investigations, it should be possible to avoid some cases of postoperative myxoedema, if due regard is paid to the antibody findings before partial thyroidectomy is performed.

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From the Department of Medicine (Head Nils Bergqvist, M. D.) and the Department of Roentgenology (Head Ingemar Hemén, M. D.) Länslasarettet, Ludvika, Sweden

Arteriovenous Pulmonary Aneurysms in Osler's Disease (Telangiectasia hereditaria haemorrhagica)

Report of four Cases in the same Family

By

Nils Bergqvist, Ingemar Hemén and Mogens Hey

At Ludvika Lasarett we have had occasion to diagnose arteriovenous pulmonary aneurysms in a family in which several members suffered from hereditary haemorrhagic telangiectasia, a condition also known as Osler Weber Rendu disease. This prompted us to examine the entire family in order to search for additional members with pulmonary aneurysm.

Initially known as hereditary epistaxis (Babington 1865) hereditary haemorrhagic telangiectasia has been recognized as a disease entity for practically a century. Rendu (1896), Osler (1901) and Weber (1907) described the condition as mainly affecting the skin and mucous membranes, and Hanes (1909) named it telangiectasia hereditaria haemorrhagica. Over the next few decades cases were reported from time to time, and a series of papers dealt with its inheritance (Teahan 1939, Bird et al. 1937). In recent years the association between hereditary haemorrhagic telangiectasia and cirrhosis of the liver has been discussed (Grung 1954) and during the last two decades pulmonary manifestations of the condition have attracted particular attention (Lindgren 1946, Lodin 1952, Grung 1954, Heyde 1954, Sammons 1959).

In 1942 the first two cases in which surgical treatment was used for arteriovenous pulmonary aneurysms were reported by Hepburn and Dauphinée.

Hereditary haemorrhagic telangiectasis is an inheritable disease transmitted by a dominant gene which is not sex-linked. Hence it is equally prevalent in males and females, the initial lesions often appearing in the early teens. It is characterized by multiple arteriovenous aneurysms mainly situated in the skin and mucous membranes. Telangiectatic dilatation of the small vessels in the skin of the cheeks, in and on the nose, at the lips, on the tongue, and beneath the fingernails is pathognomic of the condition. Cases have been described in which the patient had lesions in several organs of the body. The aneurysms often have rather thin walls and are liable to spontaneous rupture. Accordingly haemor

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rhage is a common sign and epistaxis is highly characteristic.

Anatomically the pulmonary arteriovenous aneurysm constitutes a pathological connection between an afferent artery and one or more efferent veins The capillary bed normally interposed between the vessels is absent. The communication may take the form of a fistula marked by dilatated and irregular vessels, or of a more or less completely developed aneurysmal sac with thin walls The abnormal region may be so small that it appears merely as a vessel which unlike normal vessels, does not narrow as it approaches the margin of the lung (Lodin 1952) Whilst the fistula usually connects the pulmonary artery and vein in the same lobe, it may pass from one lobe to another The artery may also be one arising from the aorta or from an intercostal artery In 20 per cent of the cases the aneurysms are said to be bilateral or to occur in two ipsilateral lobes (Sammons 1959) Apparently calcifications are very seldom present in the aneurysms they had been observed in 3 of the over 200 cases of aneurysm published (Sloan and Cooley 1953)

Although this is not a reliable rule, variations in the size of an aneurysm may be induced by Valsalva s manoeuvre and/or Müller s experiment. Aneurysms are further said to be capable of pulsating However pulsations of intrapulmonary structures may be transmitted and should be assessed cautiously even if the pulsations apparently are expansile A bruit may be present over an aneurysm.

Radiographic diagnosis of pulmonary aneurysms can be extremely difficult because they may be so minute and the vascular connections are liable to be overlooked at a routine examination. Tomography often proves very helpful. A conclusive diagnosis may be arrived at with the aid of angiography whereby even very tiny aneurysms can be visualized

Considering that in hereditary haemorrhagic telangiectasis the number of telangiectases in the skin and mucous membranes rises with age, it seems reasonable to assume that the same applies to the pulmonary aneurysms. The literature indicates that 70 per cent of all pulmonary aneurysms occur in patients under 40 They have even been observed in newborns but are on the whole rare in children (Purnel & Muras 1957) The abnormality seems to be equally prevalent among males and females. Aneurysms may be present in the lungs even when the skin and mucous membranes exhibit no telangiectatic manifestations (Sammons 1959)

When symptoms are present, either aneurysmal rupture or the intrapulmonary arteriovenous shunt is responsible. Rupture is accompanied by haemoptysis and on rare occasions by haemothorax. Rarely the haemoptysis is associated with recurrent but transient and variable cerebral manifestations hemiplegia cramps, etc. Broman (1953) described a case of this type. A man of 28 had haemoptysis which on several occasions was attended by attacks of various cerebral disturbances, such as unconsciousness cramps, temporary blindness and hemiplegia. The man had hereditary haemorrhagic telangiectasis and numerous pulmonary arteriovenous aneurysms. Broman held that cerebral air emboli are responsible for the attacks. Symptoms produced by the presence of an arteriovenous shunt take the form of more or less pronounced signs due to

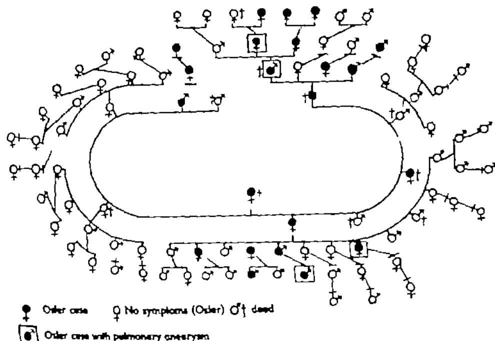


Fig. 1 Pedigree. A family (93 persons) with hereditary telangiectasis (Osler)

defective pulmonary aeration of the blood (cyanosis, hyperglobulism, dyspnoea, drumstick fingers).

So far as we are aware the only previous publication on the incidence of pulmonary aneurysms in a family with hereditary haemorrhagic telangiectasis is the one by Hodgson et al. (1959). The family comprised 231 members of whom 91 had hereditary haemorrhagic telangiectasis. Of the latter 14 (i.e. 15.4 %) had pulmonary aneurysms, 6 of these exhibiting symptoms ascribed to pulmonary aneurysms and 8 lacking symptoms, the condition being discovered at routine examinations of the chest. These persons were between 15 and 55 years of age. The aneurysm was seen to grow to twice its original size in a patient followed for 11 years.

Material

The present investigation is concerned with a family comprising 93 members (fig. 1) belonging to 5 generations. The dominant inheritance is clear. From all 5th generation members with diagnosed hereditary haemorrhagic telangiectasis the condition can be traced back through all the generations to a common maternal ancestor. We are in possession of data for all the 93 persons, enabling us to diagnose it in 22 persons. For 17 of these 22 persons the available information could be supplemented by examination. Arteriovenous pulmonary aneurysms were detected in 4 of the 17 patients. The remaining 5 persons either had died or were unable to come for an examination. Although the cases were too few to permit computation of percentages, it is interesting to note that the incidence of aneurysms among our patients was 23.5 % (as contrasted with Hodgson's 15.4 %).

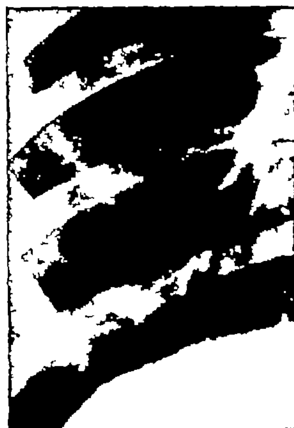


Fig 2. Case 1 The pulmonary aneurysm on the 2nd of October 1941



Fig 3 Case 1 The aneurysm some eight months later

Case reports

Brief case histories will now be given for the 4 patients with diagnosed arterio-venous pulmonary aneurysms

Case 1 E. D a woman born 1924 Her grandfather father and daughter exhibit signs of hereditary haemorrhagic telangiectasia. Since age 13 she has often had epistaxis and been anaemic. In her 17th year erythema nodosum was diagnosed and she was sent for a check-up to the local TBC unit. During pregnancies in 1954 and 1955 she suffered from haemoptysis unaccompanied by cerebral manifestations. In recent years her menses have been more abundant than before. She had an upper respiratory tract infection with stubborn coughing in November 1958. In December 1958 she was admitted to the Medical Ward of Ludvika Lasarett. Her condition was then satisfactory and there were no signs of circulatory insufficiency In the face, particularly on the cheeks, at the lips and on the tip of the tongue there were numerous pinhead-sized, darkred spots which pulsated and disappeared when pressed with a glass spatula. Heart a soft, systolic murmur heard over the apex regular heart rhythm. Blood pressure 100/65. Lungs A faint murmur synchronous with the pulse was heard in a palm-sized area of the back over the base of the right lung Blood Hb 79 RBC 4 0 million, colour index 0.98. WBC 4 100, serum-iron 34 γ% E. S R. 8 mm/1 h. Bicycle ergometry showed a work output of 600 kgm/min. The ECG was normal at rest and unchanged after exercise. Maximal breathing capacity 88.5 litres/min. (estimated normal value 89.39 litres/min.).

X ray examination of the chest On re-examining films from the TBC check when the patient was 17 one finds that on October 2nd 1941 there was slight dilatation of a vascular loop connected to the lower part of the right hilus (fig 2) Some 8 months later (the



Fig. 4. Case 1. The aneurysm sixteen years later in 1956.



Fig. 5. Case 1. Tomography of the aneurysm at the same time as in fig. 4.

had been followed-up on June 3rd 1942) this loop was more distended. The vascular loop consists of two branches attached to the hilus between whose lateral portions there is localized dilatation about as big as pea. Accordingly the lesion presents as an arterio-enous aneurysm w th an afferent artery and an efferent ein (fig. 3). Chest X-ray from 1956 show tha the aneurysm has increased in size. It consists of an upper arterial branch 1/2 cm ide and lower enous branch measuring 1 cm across. The lateral portion of the aneurysmal shunt presents as diffuse vascular dilatation up to 1 1/2 cm wide (fig. 4). Tomography proved best for localizing the lesion (fig. 5). The size of the aneurysm seemed unchanged during Valsalva manoeuvre and Muller experiment. The heart is 15.5 cm long, 11.5 cm wide, has sagittal diameter measuring 10 cm, and shows olume of 750 ml which corresponds to 450 ml per m² of body surface. The size of the heart thus falls slightly abov the range of normal variation. The configuration of the heart displays no abnormalities.

In March 1959 the patient was admitted to Falu Lasarett where angiography was per formed and the aneurysm resected.

A follow-up examination was mad t Ludvika Lasarett in September 1960. There were no cardiopulmonary symptoms. The patient still had epistaxis. Hb 63 serum iron 33 γ.

X-ray examination of the chest now disclosed that the 6th rib on the right side had been resected and the aneurysm removed. No abnormalities were visible in the lungs. The size and configuration of the heart were the same as before the operation.

Result

A woman of 36 with a family history of hereditary haemorrhagic telangiectasis. Frequent epistaxis since age 15. Telangi-

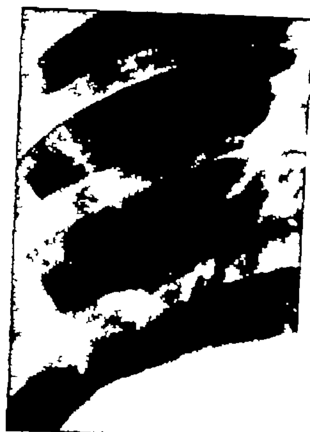


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Case 3. K. H. R. a man born 1941 whose father and paternal grandmother have hereditary haemorrhagic telangiectasia. An aunt on the father's side has pulmonary arteriovenous aneurysms (case 2). Since age 13 the patient has often had epistaxis, during the last year up to 3 times a week. Previously he was in fairly good condition but lately he becomes breathless when climbing stairs or walking uphill. Mass chest X-ray examination of the lungs in 1957 and 1958 disclosed no abnormalities. H was admitted to the Medical Ward of Ludvika Lasarett in 1959. At the time of admission the youth was in good general health. No cutaneous aneurysms were observed on the face or tongue. A few aneurysms were present on the palms, *on the right forearm and around the left shoulder.* Heart. A weak systolic murmur was heard with the point of maximum loudness over the apex. Blood pressure 125/80. A [illegible] murmur synchronous with the pulse was heard in the 5th intercostal space immediately lateral to the right midclavicular line and rough murmur synchronous with the pulse in the 5th left intercostal space one or two fingerbreadths lateral to the apex of the heart. Both these murmurs differed in character *from the systolic murmur heard* over the heart. Blood Hb 89 %, RBC 4.2 million, colour index 1.05, serum iron 47 γ. The resting ECG was normal. His work output on the bicycle ergometer was 600 kgm/min for 3 minutes.

X-ray examination of the chest. Rescrutiny of films from the mass chest examinations undertaken in 1957 and 1958 disclosed no abnormalities. X-ray examination on May 28th 1959 disclosed the presence in the anterior basal portion of the left lung of hazelnut-sized, rounded density which communicated with the hilus via pair of vessels. Describing shallow anteriorly convex arch, one of these lay nearer the chest wall and had diameter of about 1/2 cm, whilst the other took more dorsal and straighter course and had somewhat larger calibre. At the corresponding site in the anterior basal part of the right lung there is density as big as the tip of the little finger and with rather polycyclic border. It is attached to the hilus by way of two vessels, one 1/2 cm in diameter and another narrower one passing alongside it. Both abnormalities thus have the appearance characterizing an arteriovenous aneurysm. No alteration in size occurred during Valsalva's manoeuvre or Müller's experiment.

The heart is 13.5 cm long, 11.5 cm wide and 9 cm along the sagittal diameter. This yields a calculated cardiac volume of 590 ml, corresponding to 320 ml per m² of body surface. Hence the heart is not enlarged. It presented no configurational abnormalities.

In the spring of 1960 the patient was admitted to the Thoracomedical Department of Karolinska Sjukhuset in Stockholm, where angiography was performed and revealed — in addition to the aforementioned aneurysms — two further small aneurysms in the basal portion of the right lung. The arterial oxygen saturation was found to be 98 per cent at rest and 93 *per cent after exertion,* negligible diminution. Surgical treatment was considered not indicated because the patient had no symptoms from his pulmonary aneurysms and also because these were multiple and bilateral. Owing to the aneurysms and to the frequent haemorrhages from the nose, however it was deemed advisable to have the patient lumberjack retrained for another less arduous occupation.

Result

A lumberjack of 19 with a family history of hereditary haemorrhagic telangiectasia. Frequent epistaxis since age 13. A few cutaneous aneurysms on arms and back. Chest X-rays, plain films supplemented with tomograms, disclosed an aneurysm in each lung. Angiography disclosed two additional, small aneurysms in the right lung. The absence of the aneurysms in films from the previous *mass chest examinations* does not mean they had developed since then. A more likely explanation is the technical imperfections of mass chest X ray that make it inadequate for certain exacting applications. An operation was not considered indicated since the patient had no symptoms and the aneurysms were multiple and bilateral. He was advised t a 'oid heavy manual labour

ectases on cheeks at lips and on tip of tongue X ray examination disclosed a solitary aneurysm in the right lung it showed growth. The size of the heart lay slightly above the range of normal variation The aneurysm was removed by resection. When followed up after 18 months the patient's lungs seemed normal and the size of the heart was unchanged

Case 2 R. R. a woman born 1907 whose mother and grandmother had symptoms of hereditary haemorrhagic telangiectasia. Since an early age the patient has had frequent attacks of epistaxis and been anaemic. During a year or so at the age of 15 she experienced recurrent attacks, lasting up to an hour of unconsciousness with cramps and paralysis of the left side. Medical advice was not sought in conjunction with these manifestations.

Haemoptysis set in at age 18. This caused the patient to be examined at a sanatorium 1925 the chest X ray revealing a density in the left lung From then on up to 1957 the patient was examined regularly at a TBC unit. The density in the lung was thought to be a benign tumour In September 1958 she was admitted to Ludvika Lasarett for exertional dyspnoea and precordial pains.

On admittance the patient was in good condition. There were no signs of cardiac failure. On the face, particularly at the lips, there were numerous, approximately pinhead sized dark-red spots which pulsated and disappeared when pressed with a glass spatula. About ten similar spots were noted along the margins of the tongue. Heart a weak systolic murmur was heard over the entire precordium. Blood pressure 130/90 Lungs a faint murmur synchronous with the pulse was noted over a palm-sized area of the back below and lateral to the scapula. Blood Hb 51 % RBC 3.2 million, colour index 0.79, serum iron 26 γ The ECG at rest was normal.

X-ray examination of the chest. Films from TBC checks were available as far back as 1939 when the patient was 32 They disclosed on the anterior aspect of the left lung at the boundary between the superior and inferior lobes a rounded, well circumscribed density about the size of a hen's egg. A vascular branch as thick as a finger passed from the hilus to this density Above it at hilar level in the lateral part of the lung there was a further density which was pea-sized and somewhat angular Two narrow vessels passed to it from the hilus. The picture has consistently been the same. Neither Valsalva's manoeuvre nor Müller's experiment had any effect on the size of the densities. The lesions have the appearance of arteriovenous aneurysms. Measuring 12 cm in length, 10.5 cm in width and 10 cm along the sagittal diameter the heart has a volume of 530 ml, corresponding to 320 ml per m² of body surface. Thus the heart is not unduly large and it exhibits a normal configuration. More recent analyses have shown Hb values in the range 50 to 80 % The serum iron level is persistently low owing to frequent haemorrhages from the nose. At the most recent chest examination in July 1960 the pulmonary appearances remained unaltered.

Result

A woman of 53 with a family history of hereditary haemorrhagic telangiectasia. Recurrent epistaxis and anaemia since an early age Telangiectases on face, at lips and on tongue. Cerebral manifestations at 15 haemoptysis at 18. X-ray examination discloses two arteriovenous aneurysms in the left lung These have remained stationary and undergone no change in size during at least the period of 20 years for which a complete set of films has been preserved. The transient cerebral attacks the patient had when she was 15 may have been due to cerebral air embolism (cf. Broman's case) although it is unknown whether the patient had haemoptysis during these attacks. Presumably the haemoptysis at 18 was due to rupture of a pulmonary aneurysm Since the aneurysms had not increased in size during a long period of observation and since symptoms had been absent for decades, surgical procedures were deemed unnecessary

enlargement was probably not induced by its presence. The other two patients have hearts of absolutely normal size. X-ray examinations intended to reveal intrapulmonary aneurysms must be done with a contrast medium injected into the pulmonary vessels, lest any aneurysms be overlooked. Since such procedures naturally can not be adopted when, as in the present investigation, an entire family is examined, the incidence of pulmonary aneurysms among persons with hereditary haemorrhagic telangiectasis belonging to the same family must be a minimum figure.

Summary

In a family with hereditary haemorrhagic telangiectasis, 4 of 17 members in which it was diagnosed were found to have pulmonary arteriovenous aneurysms. Symptoms in the form of haemoptysis and/or circulatory insufficiency were present in 3 of these persons. In one case the aneurysm progressed considerably during an observation period of 17 years and was removed at operation.

In one of the patients in whom the condition produced pulmonary symptoms, at least once transient cerebral manifestations followed the onset of haemoptysis, suggesting that rupture of the aneurysmal wall had given rise to cerebral air embolism. Another patient with haemoptysis who had transient cerebral manifestations, but this relationship between the two phenomena has not been established.

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Case 4 E. J a man born 1902 and died 1946 whose father paternal grandmother and two daughters have symptoms of hereditary haemorrhagic telangiectasia. One of the daughters has been operated on for a pulmonary arteriovenous aneurysm (case 1) He had epistaxis frequently since early youth The daughters state the patient had red spots on the face and at the lips. Until the spring of 1926 he was on the whole in good health. Treated in Ludvika Lasarett seven times between 1926 and 1946 for epistaxis, haemoptysis, anaemia and circulatory insufficiency Drumstick fingers are mentioned in the case notes. Admitted to Ludvika Lasarett in April 1928 for massive haemoptysis. On the 6th day after admission the patient lost consciousness and had cramps and at the same time had a small haemoptysis. Afterwards he had left sided hemiparesis which slowly vanished over the next few months. X ray examination of the chest revealed dense shadows with smaller spots radiating towards both sides The films have been lost, so could not be scrutinized anew In 1946 the patient died in anaemia and circulatory insufficiency

Result

A man dying in his 44th year with a confirmed predisposition to hereditary haemorrhagic telangiectasis (father and daughters affected) Frequent epistaxis since youth, telangiectases in face and at lips, drumstick fingers, and — from age 26 — increasing circulatory insufficiency Recurrent haemoptysis during a two-year period commencing at about age 25 On one occasion haemoptysis was attended by a sudden attack of cramps, unconsciousness and temporary leftsided hemiparesis. Died in anaemia and circulatory insufficiency in 1946

Disease histories compiled from and deductions based on old case notes are obviously liable to be less than perfect Nevertheless this patient undoubtedly suffered from hereditary haemorrhagic telangiectasis. It would have been interesting to rescrutinize his X ray films but these have unfortunately not been preserved. Repeated haemoptysis and drumstick fingers are a combination suggestive of the two cardinal signs of an arteriovenous pulmonary aneurysm — haemorrhage and shunting — and make the diagnosis highly probable. Haemoptysis accompanied by an attack of cerebral manifestations makes one suspect cerebral air embolism due to rupture of an aneurysmal wall (cf Broman)

Discussion

The presence of pulmonary manifestations of hereditary haemorrhagic telangiectasis in 4 out of 17 persons — Hodgson s corresponding figures were 14 out of 91 — means that such manifestations are prevalent enough to justify X-ray examination of the lungs of all persons in whom the condition is diagnosed. Among the 4 persons with pulmonary aneurysms observed in the present investigation, one had haemoptysis, cyanosis and drumstick fingers, two others had had haemoptysis, two had attacks of cerebral manifestations suggestive of air embolism, and only one has been entirely without symptoms so far

In one of the patients the aneurysm was found to have grown considerably between her 17th and 18th year and her 34th year Consequently and because she had been troubled by haemoptysis, surgical treatment was given. In another case the aneurysms remained stationary through an observation period of 16 years from the patient s 37th to her 53rd year

The size of the heart of one of the 3 living patients lies slightly above the upper limit of normal variation The heart size was not reduced when the aneurysm had been removed and the

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Studies on the Peripheral Circulation and Metabolism in Man

II. Oxygen Utilization and Lactate-Pyruvate Formation in the Legs at Rest and during Exercise in Patients with Arteriosclerosis Obliterans

By

Lars A. Carlson and Bengt Pernow

One of the factors governing the degree of performable muscular work is the ability to increase the blood flow to meet the oxygen demands of the exercising tissue. It is generally accepted, that the fundamental disorder in arteriosclerosis obliterans is an incompetence to increase adequately the regional blood flow during leg exercise. Since, however reliable methods for estimating the blood flow during work in man are lacking we are in general limited to evaluating the arterial circulation by means of arteriography and oscillometry In the present paper however we have studied the feasibility of obtaining more dynamic estimations of the adequacy of the blood flow through the legs during exercise in patients with obliterating arteriosclerosis. We have used the previously described technique with peripheral catheterization and estimation of oxygen utilisation and of formation of lactate and pyruvate in the legs (1 2)

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First a rough estimate of the increase in blood flow through exercising legs can be obtained, if the total oxygen consumption and the femoral arteriovenous (AV) oxygen difference are known (1 3) Using this method, it was calculated that the blood flow through the legs normally increases from 0.5—1.0 liter/minute at rest to about 10 liters/minute at very high levels of exercise, which represents about 70 per cent of the cardiac output (1 3) In patients with impaired circulation at work due to mitral stenosis, the blood flow through the legs was lower than normal during work, but this fraction of the flow represented a larger proportion of the cardiac output (3)

Secondly it would appear possible to get information about the degree of cellular hypoxia by estimation of the amounts of lactate and pyruvate formed in the legs. As an inadequate blood supply will

Supported by grants from the Swedish Medical Research Council.

Table I. Data obtained in connection with leg exercise in normal human subjects and in patients with arteriosclerosis obliterans

		Controls				Patients with arteriosclerosis obliterans			
		Rest	Pulse rate during work			Rest	Pulse rate during work		
			90–110	110–130	130–145		90–110	110–130	130–145
Venous oxygen saturation percent	N	15	14	9	6	10	9	7	6
	M	68	30	29	23	66	17	15	9
	SE	2.1	1.4	1.9	0.8	2.8	2.1	1.9	1.7
	SD	8.0	5.6	5.8	1.8	9.7	4.5	5.1	4.1
	P					>0.3	<0.001	<0.001	<0.001
Arterial pH	N	10	7	4	3	6	6		5
	M	7.432	7.409	7.386	7.372	7.422	7.401		7.359
	SE	0.004	0.008			0.009	0.010		
	SD	0.010	0.017			0.020	0.019		
	P					>0.3	>0.1		
Venous pH	N	10	7	4	5	6	6	4	5
	M	7.416	7.343	7.327	7.297	7.410	7.295	7.250	7.203
	SE	0.008	0.015			0.010	0.017		
	SD	0.021	0.036			0.025	0.035		
	P					>0.1	<0.01		
Venous pO_2 mm Hg	N	10	7	4		6	6		
	M	37	23	23		39	17		
	SE	1.1	0.7			1.3	0.5		
	SD	3.3	1.8			3.1	1.2		
	P					>0.2	<0.001		
Arterial lactate mM per liter	N	15	9	7	10	10	11	8	9
	M	0.97	1.71	2.60	3.02	0.99	3.50	4.20	5.42
	SE	0.05	0.10	0.12	0.18	0.06	0.15	0.33	0.18
	SD	0.13	0.26	0.37	0.52	0.16	0.50	0.94	0.55
	P					>0.7	<0.001	<0.001	<0.001
Venous lactate mM per liter	N	15	11	9	10	10	9	7	6
	M	0.97	2.10	2.91	3.78	1.01	4.72	3.88	7.04
	SE	0.03	0.17	0.25	0.10	0.03	0.35	0.57	0.40
	SD	0.11	0.58	0.83	0.46	0.12	1.05	1.28	1.10
	P					>0.3	<0.001	<0.001	<0.001
Arterial pyruvate mM per liter	N	10	10		8	7	7		4
	M	0.11	0.18		0.21	0.11	0.19		0.20
	SE	0.01	0.01		0.02	0.02	0.03		
	SD	0.02	0.02		0.04	0.03	0.03		
	P					>0.1	>0.1		
Venous pyruvate mM per liter	N	10	10		6	7	7		6
	M	0.12	0.22		0.27	0.12	0.20		0.28
	SE	0.01	0.01		0.01	0.01	0.01		0.01
	SD	0.02	0.03		0.03	0.01	0.03		0.02
	P					>0.8	>0.1		>0.5

necessarily cause an intracellular hypoxia, such determinations might afford an objective measurement of the adequacy of the blood supply. Repeated determinations of the lactate and pyruvate concentrations in blood draining exercising muscles have, in fact, proved to give valuable information on the adequacy of cellular oxygenation. This was illustrated in a preliminary study on the lactate formation in healthy subjects (controls) and in patients with impaired peripheral circulation due to arteriosclerosis obliterans (1). The femoral venous blood lactate also increased more rapidly and maximal values were obtained at much lower work loads and pulse rates than normally. The AV lactate difference over the legs during work was much more pronounced in these patients than in the controls: this was taken as evidence of a smaller increase in blood flow than in healthy subjects (1). Such metabolic studies offer a way to estimate the degree of impairment which has previously been possible only by means of arteriography and oscillometry.

The present paper is an account of further studies on the same lines as those reported in the first paper in this series (2). Data are given on the arterial and femoral venous oxygen saturation as well as the lactate and pyruvate concentrations. Calculations of the increase in blood flow in the legs and the anaerobic metabolic rate during exercise have also been made.

Case material

The case material comprised 12 males, aged 50—62 years, with arteriosclerosis obliterans. The salient symptom was almost always unilateral intermittent claudication after walking 100—500 meters. Physical examination showed good general condition in every case, and normal blood pressure. In 10 cases the ECG was normal. Two patients had slight S—T depression at rest, accentuated during work. Chest X-rays were normal in every case. Impaired circulation in the legs was demonstrated by the absence of palpable pulsations in the dorsalis pedis and posterior tibial arteries, by oscillometry — which showed smaller pulsations in the affected leg in all cases — and by arteriography which disclosed obliteration or narrowing of the lumen of the femoral or popliteal artery.

The control series was described in the first paper in this series (2). It consisted of 10 males aged 20—37 years, and 5 aged 48—56 years. No significant difference was present between the values obtained in the two age groups and they were here treated as a single control series.

Procedure and methods

Catheters were inserted percutaneously into the brachial artery and into one or both femoral veins. The femoral vein was punctured about 2 cm below the inguinal ligament, and the catheter inserted about 8 cm distally. Exercise was performed sitting on a bicycle ergometer with one leg working and the other resting. Work was started with a load of 150 kpm/minute and increased every 5 minutes by 150 kpm/minute. The pedal was specially arranged with a spring which made the return of the pedal passive. In 8 cases, the work was repeated with the other leg, after 30 minutes rest. Exercise was continued until the patient complained of pain in the working leg. The pulse rate was calculated from the ECG.

Arterial and venous blood samples were drawn simultaneously at rest and repeatedly during and after work. All values obtained at rest refer to the supine position, and those obtained during exercise to the sitting position.

The methods for determination of oxygen saturation, pH and lactate and pyruvate concentrations in blood were identical with those used in the previous paper (2). Determination of oxygen uptake was performed by the Douglas bag technique. Expired air was collected during 5 minutes at rest, and from the 2nd to 5th during work.

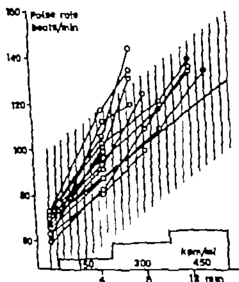


Fig. 1. Pulse rate at rest and during one-leg exercise in patients with arteriosclerosis obliterans. The straight line represents the regression line of the control series. The equation is $y = 67.60 + 0.132x$, with standard error of estimate (Sy) for $y = 15.6$ and coefficient of correlation ( ) = 0.81. The hatched areas represent $\pm$ 2 standard errors of the estimate.

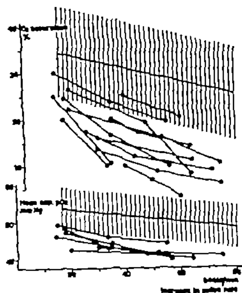


Fig. 2. Femoral venous oxygen saturation in % (upper diagram) and calculated mean capillary pO in mm Hg (lower diagram) during one-leg exercise, correlated to rise in pulse rate from the resting value. Normal regression line equations $y = 35.35 - 0.138x$, $Sy = 4.4$ $= -0.56$ (upper diagram), and $y = 33.42 - 0.058x$, $Sy = 2.7$ and $= -0.44$ (lower diagram). The hatched areas represent $\pm$ 2 standard errors of the estimate. The figures refer to the "bad" leg.

vious at heavier loads (7 359 and 7.203 as mean values for 5 subjects of the patient material and 5 control subjects respectively at pulse rate 130—145)

Blood oxygen tension

The oxygen tension of arterial and venous blood was calculated from HbO and pH according to Dill, Edwards and Consolazio (4). As in the controls, the arterial pO was unchanged or slightly decreased during work. The femoral venous pO at rest was 33—45 mm Hg (mean 39) the same as in the controls. During exercise, the venous pO decreased markedly to significantly lower values than in the controls (11—19 mm Hg mean 16 as compared to 20—25 mm Hg, mean 23 $p < 0.001$) Five minutes after work, the venous pO had risen to a mean 47 mm Hg. This value was significantly higher than at rest before work ($p < 0.001$) but within the same range as in the healthy subjects ($p > 0.7$) The mean capillary pO was calculated according to Barcroft's ( ) formula

$$\text{venous pO} + \frac{\text{arterial pO} - \text{venous pO}}{3}$$

the results are given in fig 2.

Arterial and venous lactate concentration

The lactic acid concentration at rest was 0.75—1.20 mM/liter (mean 0.99) in the arterial blood, and 0.80—1.23 mM/

Table I (cont.)

		Controls				Patients with arteriosclerosis obliterans			
		Rest	Pulse rate during work			Rest	Pulse rate during work		
			90–110	110–130	130–145		90–110	110–130	130–145
Arterial XL mM per liter	N		7	7			7	5	
	M	0	0.16	0.98		0	1.26	1.94	
	SE		0.04	0.06			0.26	0.32	
	SD		0.09	0.15			0.73	0.92	
	p						<0.001	<0.001	
Venous XL mM per liter	N		9	8		0	7	7	
	M	0	0.26	1.03			2.20	2.97	
	SE		0.05	0.07			0.44	0.52	
	SD		0.15	0.20			1.15	1.38	
	p						<0.001	<0.001	

N = number of observations M = mean SE = standard error of the mean, SD = standard deviation.
p = p value for the differences between controls and patients.

Results

Pulse rate during work

The increase in pulse rate during work was within normal limits in 9 cases, whereas in 3 the end values were beyond 2 standard errors of the mean for the controls. For the whole group the mean pulse rate at 300 kpm/minute was 114 the corresponding figure in the controls being 100 beats/minute (fig 1)

Oxygen saturation of arterial and venous blood

The oxygen saturation of arterial blood was 96—99 per cent (mean 97) at rest in every case, and remained unchanged during and after work The oxygen saturation of femoral venous blood was also within normal limits (50—78 per cent, mean 66) at rest. At the first work load the venous oxygen saturation was significantly lower than in the controls. During continuous exercise, a steady decrease took place in oxygen saturation, the lowest values always being recorded at the highest load. At the maximal intensity of work, the mean femoral venous oxygen saturation was 12 per cent, as compared to 23 per cent at a corresponding pulse rate in the controls (fig 2) This difference is highly significant ($p < 0.001$).

Five minutes after ending work, the venous oxygen saturation had risen to values significantly higher than before work (mean 78, as compared to 66 per cent $p < 0.001$) This rise was within the same range as that in the controls (mean 78 as compared to 79 per cent $p > 0.5$)

Blood pH

The values obtained for the pH of arterial and femoral venous blood at rest did not differ significantly from the controls. During exercise, the pH of the venous blood decreased in the arteriosclerotic patients to values which were significantly lower than in the normal subjects. This was true already at a low degree of work, and was even more ob-

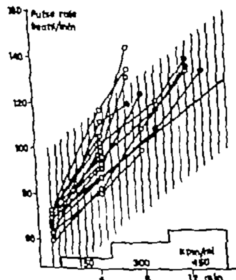


Fig. 1. Pulse rate at rest and during one-leg exercise in patients with arteriosclerosis obliterans. The straight line represents the regression line of the control series. The equation is y = 67.60 + 0.132 x, with standard error of estimate (Sy) for y = 13.0 and coefficient of correlation ( ) = 0.86. The hatched areas represent ± 2 standard errors of the estimate.

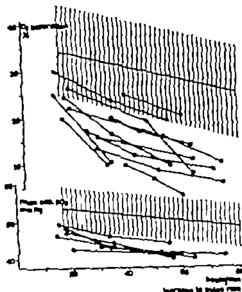


Fig. 2. Femoral venous oxygen saturation in % (upper diagram), and calculated mean capillary pO in mm Hg (lower diagram), during one-leg exercise, correlated to rise in pulse rate from the resting value. Normal regression line equations y = 35.35 — 0.158 x, Sy = 4.4, = — 0.56 (upper diagram) and y = 33.42 — 0.038 x, Sy = 2.7 and = — 0.44 (lower diagram). The hatched areas represent ± 2 standard errors of the estimate. The figures refer to the "bad" leg.

vious at heavier loads (7.359 and 7.203 as mean values for 5 subjects of the patient material and 5 control subjects respectively at pulse rate 130—145).

Blood oxygen tension

The oxygen tension of arterial and venous blood was calculated from HbO and pH according to Dill, Edwards and Consolazio (4). As in the controls, the arterial pO was unchanged or slightly decreased during work. The femoral venous pO at rest was 33—45 mm Hg (mean 39), the same as in the controls. During exercise, the venous pO decreased markedly to significantly lower values than in the controls (11—19 mm Hg, mean 16, as compared to 20—25 mm Hg mean 23, $p < 0.001$). Five minutes after work, the venous pO had risen to a mean 47 mm Hg. This value was significantly higher than at rest before work ($p < 0.001$) but within the same range as in the healthy subjects ($p > 0.7$). The mean capillary pO was calculated according to Barcroft's (5) formula

$$\text{venous pO} + \frac{\text{arterial pO} - \text{venous pO}}{3}$$

the results are given in fig. 2.

Arterial and venous lactate concentration

The lactic acid concentration at rest was 0.75—1.20 mM/liter (mean 0.99) in the arterial blood, and 0.80—1.23 mM/

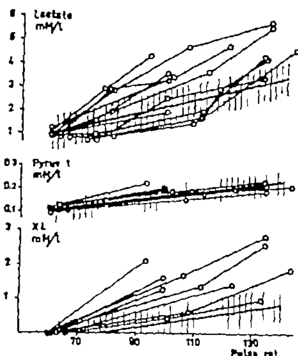


Fig 3. Arterial lactate, pyruvate and "excess of lactate" (XL) in mM/liter at rest and during one leg exercise correlated to relative working intensity (pulse rate during work) The straight lines represent the regression line of the control series, obtained from the following equations lactate $y = 0.035x - 1.58$, $Sy = 0.36$, $= 0.89$ pyruvate $y = 0.0018x - 0.019$ $Sy = 0.024$, $r = 0.83$ XL $y = 0.014x - 1.14$ $Sy = 0.{\ldots}6$ $r = 0.74$ The hatched areas represent $\pm$ 2 standard errors of the estimate. The figures refer to the "bad" leg

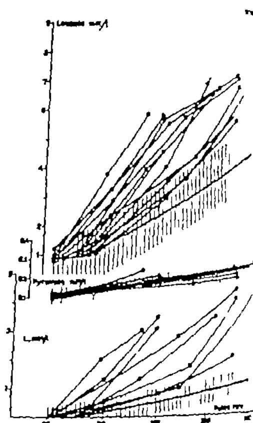


Fig 4 Femoral venous lactate, pyruvate and "excess of lactate" (XL) in mM/liter at rest and during one-leg exercise correlated to relative working intensity (pulse rate during work) Normal regression line equations lactate $y = 0.050x - 2.60$, $Sy = 0.48$, $r = 0.87$ pyruvate $y = 0.0019x - 0.034$ $Sy = 0.026$, $r = 0.79$ XL $y = 0.017x - 1.23$, $Sy = 0.17$ $r = 0.8.$ The hatched areas represent $\pm$ 2 standard errors of th estimate. The figures refer to the "bad" leg

liter (mean 1 01) in the femoral venous blood These values were within the same range as those in the controls. No significant difference could be demonstrated between the arterial and femoral venous lactate concentrations, when calculated on the individual differences.

During work, a much more rapid increase in arterial as well as in venous lactate was generally observed in the arteriosclerotic patients than in the controls. Thus at the highest work intensity (pulse rate 120—140 beats/minute) the mean lactate concentration in the arterial blood was 5 42 mM/liter as compared to 3 62 mM/liter at the same relative work intensity in the controls. This difference is highly significant ($p < 0.001$). The corresponding values for the femoral venous lactate were 7 04 and 3 78 mM/liter respectively ($p < 0.001$) Although the maximal lactate values were not, as a rule, higher in the patients with impaired circulation than in the healthy subjects, these values were recorded in the former cases at a much lower relative intensity of work (figs. 3 and 4)

A rapid increase in the venous-arterial XL difference was observed during work,



Fig. 5. Difference between "excess of lactate" (XL) in femoral venous and arterial blood, at rest and during exercise, correlated to pulse rate. Normal regression line equation: y = 0.0050 x — 0.1526, S_y = 0.098, = 0.45. The hatched areas represent ± 2 standard errors of the estimate.

the highest value recorded amounting to 2.25 mM/liter (fig. 5).

In the healthy subjects, inappreciable differences were noted when the same exercise was performed first with one leg and then with the other, which agrees with earlier experience (6). When the patients with claudication performed the same type of work, a considerable difference was observed in all but one case, between the arterial and femoral venous lactate concentration in the two legs. The highest lactate concentrations were invariably recorded in the more affected leg, as judged by the subjective findings, arteriography and oscillometry. In one case the difference at 300 kpm/minute, amounted to 7.0 mM/liter on the venous side (fig. 6).

Five minutes after the end of exercise, the mean venous lactate concentration had decreased to 3.67 mM/liter, which did not differ significantly from the value in the controls ($p > 0.2$).

Arterial and venous pyruvate concentration

The pyruvate concentration at rest was 0.08—0.16 mM/liter (mean 0.13) in arterial blood, and 0.08—0.14 mM/liter (mean 0.12) in femoral venous blood. This difference is not significant. During

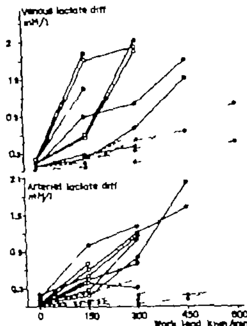


Fig. 6. Differences between femoral venous lactate (upper diagram) and arterial lactate (lower diagram) in the two legs, when exercised separately in patients with mainly unilateral claudication (—) compared to the same difference in 4 normal subjects (— — —). In the latter cases, the differences in lactate are given in the positive direction.

exercise, the arterial pyruvate concentration increased to a mean 0.17 mM/liter and the venous concentration to a mean 0.20 mM/liter at the end of work (pulse rate 120—140/minute). This increase was within the same range as that in the controls ($p > 0.1$) (figs. 3 and 4).

After work, the femoral venous pyruvate increased further and after 5 minutes reached a mean value of 0.42 mM/liter which does not differ significantly from that in the controls.

Lactate-pyruvate ratio

The lactate-pyruvate ratio was expressed as excess of lactate (XL) according to Huckabee's (8) formula

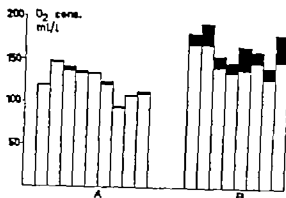


Fig 7 Total metabolism (TM) in ml oxygen consumed per liter of blood in the leg during exercise. TM is indicated by the total height of each column, and divided into aerobic metabolism (white bars) i. e., AV oxygen difference and anaerobic metabolism (black bars) obtained from the AV—XL difference by multiplying by the factor 11.2 ml of oxygen/mM of XL (7) The exercise was performed at comparable relative work intensities in both groups (pulse rate 110—130/min)

$$XL = (L_a—L_o) — (P_a—P_o) \frac{(L_o)}{P} \text{ where}$$

L and L_a represent the lactate concentration at rest and during the actual load respectively and P and P_a the corresponding values for pyruvate. As shown in figs 3 and 4 a significant difference was present between the two groups, the rise being more rapid in the pathologic cases. Since the pyruvate concentration increased only slightly during exercise, the increase in XL was almost parallel to that of lactate. This applied on both the arterial and the venous side (figs. 3 and 4)

The anaerobic fraction of the metabolism (AM) was derived from the venous-arterial XL difference, by multiplying by the factor 11.2 ml of oxygen/mM of XL (0.5 mM O /mM lactate formed from pyruvate in excess) (7) In fig 7 AM is given as a fraction of the total metabolism (TM) obtained by adding AM to the AV oxygen difference in each case During work, AM as a percentage of TM was 5.2—15.1 (mean 10.3) in the arteriosclerotic patients and 0.6—3.4 (mean 1.9) in the controls the difference being highly significant ($p < 0.001$) at comparable work intensities (pulse rate 120—140)

In four patients, the increase in *blood flow* through the exercising leg was calculated from the oxygen consumption and the oxygen utilization in the tissue (femoral AV oxygen difference) In this calculation, it was assumed that the increase in consumed oxygen above the resting level was due entirely to increased utilization in the exercising tissue. This approximation was used only when the arteriosclerotic patients were compared to the controls, since it was assumed that the oxygen consumption in tissues other than the exercising muscles was equal at the corresponding loads in the two groups of subjects. According to Fick's formula the blood flow through the leg was therefore $\frac{V_{O_{2a}} — V_{O_{2o}}}{A — VO \ diff.}$, where $V_{O_{2a}}$ is the oxygen uptake at the actual load and $V_{O_{2o}}$ the corresponding resting value.

The results of these calculations are seen in table II and fig 7 and indicate a smaller increase in blood flow during work in the patients with impaired peripheral circulation. In three cases, a difference was also observed in blood flow between the two legs, when exercised separately with the smaller increase in flow invariably in the clinically more impaired leg

Mechanical efficiency

The mechanical efficiency of the arteriosclerotic patients was 22 (19—27) per cent (mean and range for all loads) which was somewhat higher than in the

Table II. Regional arterio-venous oxygen difference, increase in oxygen consumption and regional blood flow during sitting leg exercise in normal human subjects and patients with arteriosclerosis obliterans

Case	Age years	Leg working	Work load kpm/min.	Pulse rate beats/min.	O_2-cons. ml/min.	AV-O_2 diff. ml/l	Blood flow exerc. leg. l/min.	Results of clin. invest. More impaired circ. as judged from oscillometry (O) arteriography (A)
A. Normal subjects								
E. B.	48	Left	150	87	510	132.9	4	Normal
			450	129	1,214	137.2	8	
			600	150	1,830	170.2	11	
M. L.	51	Left	300	111	758	113.2	7	Normal
			450	132	1,021	119.0	9	
			600	159	1,536	124.8	12	
K. F.	56	Left	450	134	913	122.0	8	Normal
			900	158	1,752	126.5	13	
S. G.	53	Left	300	78	687	123.0	6	Normal
			900	150	2,044	160.9	13	
O. N.	46	Left	150	98	524	143.6	4	Normal
			300	115	830	148.1	6	
			600	174	2,083	163.8	13	
L. N.	25	Left	300	90	861	128.1	7	Normal
			450	165	1,295	129.8	9	
			750	158	1,973	140.9	14	
R. E.	31	Left	150	72	478	113.0	4	Normal
			300	82	740	131.7	6	
			600	108	1,334	150.0	11	
B. Arteriosclerotic patients								
E. L.	59	Right	150	109	465	166.2	5	Left (O A.)
			300	144	791	174.8	5	
		Left	150	115	326	181.3	2	
			300	144	567	183.4	3	
E. A.	54	Right	150	83	341	126.3	3	Left (O A.)
			450	132	833	130.9	6	
		Left	150	99	271	138.4	2	
V. A.	60	Right	300	93	684	186.7	4	Right (O A.)
		Left	300	99	756	189.3	5	
S. W.	61	Right	150	81	501	167.0	2	Right (O A.)
			300	111	618	179.7	3	
		Left	150	75	341	113.8	3	
			300	108	768	130.9	6	

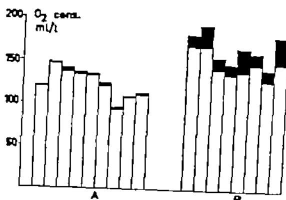


Fig 7 Total metabolism (TM) in ml oxygen consumed per liter of blood in the leg during exercise. TM is indicated by the total height of each column, and divided into aerobic metabolism (white bars) i. e., AV oxygen difference and anaerobic metabolism (black bars) obtained from the AV—XL difference by multiplying by the factor 11.2 ml of oxygen/mM of XL (7) The exercise was performed at comparable relative work intensities in both groups (pulse rate 110—130/min)

$$XL = (L - L_a) - (P_a - P_a) \frac{(L_a)}{P} \quad \text{where}$$

L and L_a represent the lactate concentration at rest and during the actual load respectively and P and P_a the corresponding values for pyruvate. As shown in figs. 3 and 4 a significant difference was present between the two groups, the rise being more rapid in the pathologic cases Since the pyruvate concentration increased only slightly during exercise, the increase in XL was almost parallel to that of lactate. This applied on both the arterial and the venous side (figs. 3 and 4)

The anaerobic fraction of the metabolism (AM) was derived from the venous-arterial XL difference, by multiplying by the factor 11.2 ml of oxygen/mM of XL (0.5 mM O_2/mM lactate formed from pyruvate in excess) (7) In fig 7 AM is given as a fraction of the total metabolism (TM) obtained by adding AM to the AV oxygen difference in each case During work, AM as a percentage of TM was 5.2—15 1 (mean 10.5) in the arteriosclerotic patients and 0.6—3.4 (mean 1 9) in the controls the difference being highly significant ($p < 0.001$) at comparable work intensities (pulse rate 120—140)

In four patients, the increase in *blood flow* through the exercising leg was calculated from the oxygen consumption and the oxygen utilization in the tissue (femoral AV oxygen difference) In this calculation it was assumed that the increase in consumed oxygen above the resting level was due entirely to increased utilization in the exercising tissue. This approximation was used only when the arteriosclerotic patients were compared to the controls since it was assumed that the oxygen consumption in tissues other than the exercising muscles was equal at the corresponding loads in the two groups of subjects. According to Fick's formula, the blood flow through the leg was therefore $\frac{V_{O_{2a}} - V_{O_2}}{A - VO_2 \text{ diff.}}$, where $V_{O_{2a}}$ is the oxygen uptake at the actual load and V_{O_2} the corresponding resting value.

The results of these calculations are seen in table II and fig 7 and indicate a smaller increase in blood flow during work in the patients with impaired peripheral circulation. In three cases, a difference was also observed in blood flow between the two legs, when exercised separately with the smaller increase in flow invariably in the clinically more impaired leg

Mechanical efficiency

The mechanical efficiency of the arteriosclerotic patients was 22 (19—27) per cent (mean and range for all loads) which was somewhat higher than in the

cent (mean value) was recorded in femoral venous blood during heavy work (2) In the patients in the present series, on the other hand, significantly lower venous oxygen saturation values were observed during work, the lowest value recorded being 4 per cent (fig. 2) Almost complete extraction of the available oxygen from the blood during exercise has, in fact, also been observed in patients with severely impaired cardiac-output responses to exercise (3)

The aforementioned reserve mechanism could not, however fully compensate for the insufficient increase in blood flow during work. This fact was clearly indicated by the more rapid increase in "excess of lactate" of the blood during work than that in the healthy subjects. High blood lactate indicates a high intracellular lactate concentration (10) which might be due either to a greater formation of lactate, or to inadequate removal of lactate from the tissue because of the lesser increase in blood flow The present study indicates that both these phenomena are present. The greater XL concentration as well as arterial lactate in the arteriosclerotic patients implies a greater formation of lactate during work in these patients than in the normal subjects. This is one of the advantages of the XL calculation over mere determination of total venous lactate concentration since the latter is dependent both on the rate of formation and on the amount of blood to which the intracellulary formed lactate is delivered

A quantitative estimate of the anaerobic metabolism (AM) in patients with impaired peripheral circulation can also be obtained by calculating AM as a percentage of TM (7) (fig 7) At a working intensity resulting in a pulse rate of 110—130 beats/minute, the mean value was 10.3 per cent, as compared to a mean 1.9 per cent in the controls. As can be inferred from fig 7 the total metabolism (TM) *per liter* of blood was greater in the arteriosclerotic patients than in the controls. However since the increase in blood flow during work was significantly lower in the former (fig 8) the total metabolic rate *per unit time* (ml of oxygen/minute) was actually much lower

Immediately after work, the venous oxygen saturation rose to about the same level as in the controls. The XL curves were also identical in both groups. This indicates that no difference is present between the groups with respect to the time required for restitution of normal metabolism after anaerobic work. In addition, the results indicate that it is necessary to study the changes occurring during work for evaluation of the circulatory impairment. It is, however interesting to note that, in patients with cyanotic heart disease, the lactate-pyruvate ratio was still significantly raised 15 minutes after mild exercise, whereas at rest no significant difference was present in this respect between such patients and healthy subjects (11)

The increase in arterial lactate concentration is thus significantly more rapid in patients with impaired peripheral circulation than in healthy subjects. Consequently this estimation might be used as a simplified objective test of the ability to adjust the peripheral blood flow to the oxygen demand during exercise. The results presented in this paper show that it is unnecessary to withdraw venous blood for this purpose. It is probable that this functional test can be further simplified, i. e. it can be confined to determination of the lactic acid concentration in finger-tip blood during exercise. Ob-

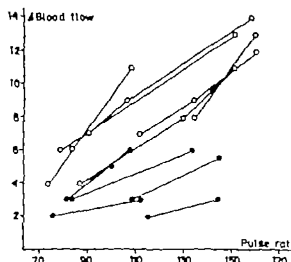


Fig 8 Increase in calculated blood flow (liter/min) during one-leg exercise in patients with arteriosclerosis obliterans (—) compared t healthy subjects (o — o) The values are plotted against the pulse rate during work. For calculations, see text.

controls i. e. 19 (15—24) It was calculated from the formula

$$\frac{\text{work performed} \times 100}{(\text{total energy exchange}) - (\text{basal energy exchange})}$$

where the energy exchange was calculated from the oxygen uptake assuming the caloric value of oxygen to be 4 9 cal./liter

Discussion

The main difference between the arteriosclerotic patients investigated in this paper and the healthy subjects previously presented (2) seems to be the inability of the former to increase the peripheral blood flow *during work in* proportion to the increased oxygen demand. However two of the patients also had a pathologic ECG reaction during work, indicative of coronary arteriosclerosis. It is therefore possible that they had impairment of the central circulation as well which *explains the more* rapid increase in pulse rate during work (fig 1) This difference can, however be almost eliminated by using the increase in pulse rate as an index of the degree of work performed (9) The results as presented in the figures have therefore generally been plotted against the relative working intensity expressed as increase in heart rate during work. If the data are plotted against the absolute work (kpm per min) the differences between the normal subjects and the arteriosclerotic patients will be even greater

When comparing cases with normal and impaired peripheral circulation it cannot be assumed *a priori* that the relation between muscular and non-muscular blood in the femoral vein is the same in both groups. It cannot be ruled out that, in patients with impaired circulation due to arterial occlusion, the cutaneous blood flow is increased in relation to the muscular flow The fact that a normal *oxygen saturation* was *found* at rest in the patients studied in this paper does not, therefore necessarily rule out that already at rest more oxygen was extracted from the muscular blood than in healthy subjects.

During work it is probable that the *composition of the femoral venous blood* — with respect to its anatomic distribution — is more comparable in the two groups. It is obvious that when the organic obstruction makes an adequate increase in blood flow impossible at work, the oxygen utilization is increased. In *peripheral* oxygen uptake from the blood there is apparently a reserve which — under normal conditions — is never completely utilized even on maximal muscular work (2) but which can be used when the raised oxygen demand can not be compensated for by an increased regional blood flow Thus, in healthy subjects, an oxygen saturation of 23 per

cent (mean value) was recorded in femoral venous blood during heavy work (2) In the patients in the present series, on the other hand, significantly lower venous oxygen saturation values were observed during work, the lowest value recorded being 4 per cent (fig 2) Almost complete extraction of the available oxygen from the blood during exercise has, in fact, also been observed in patients with severely impaired cardiac-output responses to exercise (3)

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viously determination of the AV lactate difference in the exercising leg is a far more sensitive test.

In these patients, the difference between the increase in peripheral blood flow in the two legs — *as reflected by the* great difference in arterial and venous lactate concentration at the same intensity of work (fig. 6) — was in good agreement with the subjective complaints, and the clinical evaluation of circulatory impairment. In most cases, however, the increase in lactate concentration was also abnormally rapid on work with the "better" leg. This was found to apply even in legs with no arteriographic changes, with apparently normal oscillographic tracings, and with no discomfort on walking, in view of the far greater circulatory impairment in the other leg.

Summary

1. The impairment of peripheral circulation was studied at rest, during and after leg exercise in 12 patients with intermittent claudication due to arteriosclerosis obliterans. By indwelling catheters arterial and femoral venous blood could be withdrawn both at rest and during work.

2. At rest the femoral venous oxygen saturation was within the normal range. During exercise, it was significantly lower than in the controls. Five minutes after work, the oxygen saturation had reached the normal level.

3. The calculated venous and mean capillary oxygen pressures fell to values lower than normal during work.

4. The arterial and venous lactate concentrations were normal at rest but increased rapidly during work, reaching maximal values at lower pulse rates than in the normals. Since the increase in pyruvate was similar in these patients to that in the controls, the "excess of lactate" increased much more rapidly than normally. This is taken to indicate an inadequate oxygen supply to the exercising muscles even at a low working intensity. The changes in lactate and pyruvate after work were similar to those in the controls.

5. The increase in blood flow through the exercising tissue, as calculated from the oxygen consumption and arteriovenous oxygen difference, was much lower in these patients than in the controls. The calculated increase in blood flow during exercise was lower in the leg judged to be more affected on the basis of the history, arteriography and oscillometry. The increase in both arterial and venous lactate, as well as in the venous-arterial lactate difference, was also more rapid in this leg during exercise.

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Thrombo-Embolism in Patients with Total Proconvertin (Factor VII) Deficiency

A Report on two Cases

By

H. C. Godal, K. Madsen and R. Nissen-Meyer

This report concerns two patients with total deficiency of proconvertin (factor VII) who experienced thromboembolic complications following surgery.

Case 1

The patient was a 29-year-old farmer and timber worker (O. L. G.). Tonsillectomy had been carried out in 1947 without abnormal bleeding.

Since 1957 he suffered from peptic ulcer. I June 1959 perforation occurred and he was admitted to the surgical department of Hedmark County Hospital. The perforation was closed. Prior to operation, the hemoglobin concentration was only 55 per cent, and 1,000 ml of whole blood were given during the operation. Much blood was found in the abdomen, but there was no excessive bleeding during or after the operation.

The postoperative course was uneventful until the 12th day, when the patient was suddenly stricken by pain in the left side of the chest followed by cough and blood-stained sputum. The temperature rose to about 39° C. X-ray revealed a minor density of the central part of the left lung. Pulmonary embolism was diagnosed, a first dose of dicoumarol was given and a blood sample taken for prothrombin determination. The P P value, however, was found to be below 5 per cent of normal, and anticoagulant treatment therefore was immediately discontinued. The further course was uneventful, the temperature fell to normal in 7—8 days, and a week later he was discharged. No signs of peripheral thrombosis were observed. The low P P value showed no response to vitamin K intravenously.

Some months later the patient was examined at the Institute for Thrombosis Research, University Hospital, Oslo, and a total lack of proconvertin was revealed. The hemostasis and coagulation mechanisms were otherwise normal as seen from table I. The patient denied abnormal bleedings, in spite of frequent traumas as a timber worker. A study of his family revealed that his two brothers (J. F. and G. F.) had complete lack of proconvertin, the father had a partial deficiency, whereas the mother (O. F.) and his sister (L. F.) had normal concentrations of proconvertin (table II). None of them had experienced abnormal bleedings.

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Table I Laboratory findings

Type of test	Normal range	Case 1	Case 2	References for methods
Bleeding time	5—11 min.	9 min.	10.5 min.	Borchgrevink & Waaler (1958)
Tourniquet test	< 20 petech.	3 pet.	12 pet.	Stefanini & Dameshek (1955)
Whole blood clotting time	2—5 min.	3.5 min.	3 min.	Hjort & Stormorken (1957)
Thromboplastin time	12.5—14 sec.	87 sec.	91 sec.	Borchgrevink & Waaler (1958)
P P value	75—125 %	< 5 %	< 5 %	Owren & Aas (1951)
Prothrombin	—	98 %	70 %	Hjort, Rapaport & Owren (1955)
Proconvertin	—	< 1 %	< 1 %	Aas (1952)
Proaccelerin	—	100 %	110 %	Aas (1952)
Fibrinogen (mg %)	200—400	287	236	Jacobsson (1955)
Anti-hemophilia A factor	—	128 %	ca. 300 %	Egeberg (1961)
Anti-hemophilia B factor	—	130 %	132 %	Egeberg (1961)
Anti-hemophilia C factor	—	82 %	144 %	Egeberg (1961)
Fibrinolysis	No	No	No	
Platelet count per mm	200,000—400.000	289 000	304 000	Nygaard (1953)
Clot retraction after 24 hours	> 6.5 cm	8.3 cm	6.9 cm	Voss (1958)

Table II P P proconvertin- and prothrombin values in plasma from members of the family (case 1)

	Brother (J F)	Brother (G F)	Sister (L F)	Mother (O F)	Father (L. F)
	%				
P P value	5	7	115	87	50
Proconvertin	< 1	< 5	150	96	45
Prothrombin	84	120	100	150	98

Case 2

The patient was a 75-year-old housewife. There were no known bleeders in the family Prior to the actual disease, she had been healthy

January 1961 the patient was admitted to Lillehammer hospital because of bleeding An ulcerating carcinoma vulvae with metastases to the inguinal lymph nodes was found and she was transferred to the Norwegian Radium Hospital, Oslo.

After transfusion of 1 000 ml of whole blood, vulvectomy removal of the inguinal lymph nodes and bilateral resection of vena saphena were carried out. An additional transfusion of 1 000 ml of whole blood was given.

On the 18th postoperative day the left leg became tender and swollen, with the characteristic signs of thrombophlebitis. Anticoagulant treatment was considered but not started because the P P level was found to be 5 per cent of normal. Plasma, examined at the Institute for Thrombosis Research, revealed a total lack of proconvertin. The coagulation and hemostasis mechanisms were otherwise normal (table I) As in the first patient, injections of vitamin K did not correct the clotting defect. No study of the patient's family was carried out.

Discussion

It is a common experience that only patients with *total* lack of proconvertin

are apt to bleed, and even in these cases, the bleeding tendency is moderate (Alexander Goldstein Landwehr & Cook 1951 Owren 1952 Aas 1952 Alexander 1955 Kowalski, Latallo & Niewarowski 1958 Voss & Waaler 1959). The patients studied here did not demonstrate any bleeding tendency and major surgical operations were carried out without abnormal bleeding. These observations might suggest that proconvertin plays a modest role in maintenance of a normal hemostasis.

There might be a slight possibility that the pulmonary episode in the first patient was due to pneumonia, but both the clinical picture and the X-ray findings were very suggestive of an embolism. There seems to be no doubt about the diagnosis of thrombophlebitis in the second patient. Thrombo-embolic disease includes an intravascular coagulation process. Among the known clotting factors, proconvertin occupies a unique position, since this factor is active only in the presence of tissue thromboplastin. The occurrence, with total lack of proconvertin, of thromboembolic episodes therefore suggest that postoperative venous thrombosis may occur independently of the release of tissue thromboplastin into the circulation.

Summary

Two patients with total proconvertin deficiency developing thromboembolic episodes following surgery are reported.

The significance of these observations is discussed.

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Fulminant Idiopathic Pulmonary Haemosiderosis

Report of one Case

By

Claes Grill, Sandor Szögyő and Hugo Boogren

Idiopathic pulmonary haemosiderosis (pr. haemosiderosis pulmonum essentialis) is a relatively rare disease, characterized by intrapulmonary haemorrhage. The haemorrhages are intra-alveolar and originate in the lung capillaries in the inter alveolar septa. In the beginning the blood cells escaping from the capillaries are phagocytosed. But on recurrence of the haemorrhages, the phagocytes are soon unable to cope with the situation, with the result that haemosiderin is deposited locally and diffusely in the lung parenchyma. Small amounts may also be deposited in the regional lymph nodes and occasionally in the liver and spleen.

In Scandinavia the disease has been described by Gellerstedt (1) 1939 Waldenström (2) 1944 Selander (3) 1944 Ekenmark & Kjellberg (4) 1948 Agner et al. (5) 1951 Gluck (6) 1955 Halvorsen (7) 1956 and Holm (17) 1960. Until the beginning of the nineteenfifties it was believed that the disease occurred almost exclusively in childhood and rarely in early adult or middle age. Since then, however an increasing number of adult cases have been described. In 1960 Bronson (8) was able to collect 37 adult cases from the literature, to which he added one of his own. In addition to those collected by Bronson, a few others have been published in languages other than English or German (Weber et al. (9) 1957) Denson (10) 1960 also reported a further case of idiopathic pulmonary haemosiderosis, which was fulminant.

As a rule th disease runs a chronic course with alternating exacerbations and remissions for several years. According to Bronson (8) the mean duration of survival after the initial symptoms in adults is about 3 1/2 years. So far only 3 cases of fulminant idiopathic pulmonary haemosiderosis have been described (Chatgidakis (11) 1955 Smith & Flenberg (12) Denson (10) 1960)

Below a description is given of an acute fulminant adult case of idiopathic pulmonary haemosiderosis.

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Fig 1



Fig 2

Figs. 1 and 2. Chest X ray 1 Frontal. 2 Sagittal. Widespread, diffuse dissemination of miliary partly coalescent, parenchymal densities.

Case report

On July 6 1960 a man, aged 25, presented himself at the Department of Medicine, Borås Lasarett, complaining of 5 days shortness of breath. Dyspnoea was his only complaint. He reported that he had until then felt well, but on July 1 he suddenly felt breathless while playing football, and the shortness of breath had persisted after the match. He added that he had not had such an attack before. The shortness of breath persisted, but he was still able to carry on with his work as a storekeeper On the second day the patient sought advice from a general practitioner who was not able to find anything of note the patient had no pain, no cough, no expectoration though he did report that the heart seemed to be thumping The only positive findings on admission of the patient to the Department of Medicine on the 5th day were slight resting dyspnoea and a few crepitant râles over the bases of the lungs.

No haemoptyses occurred during the patient's stay in Hospital, but his relatives reported later that one month before the onset of dyspnoea and on one occasion during a previous football match, the patient had ejected blood from the mouth. They also reported that he had often had a cold with cough during the last 6 months.

The only laboratory finding of interest was slight anaemia (Hb 68 % RBC 3 7 mill.) and a serum bilirubin value of 1.3 mg/100 ml.

Chest X-ray on admission revealed widespread diffuse dissemination of miliary partly coalescent parenchymal densities (figs. 1 and 2) The changes were most marked centrally and decreased in intensity towards the periphery to leave the most peripheral parts of the lungs unaffected. No enlargement of the hilus or substantial enlargement of the heart could be demonstrated.

The patient's condition remained unchanged for the first two days, but on July 9 he became worse. During the course of the day dyspnoea became increasingly severe with rapid shallow breathing Body temperature pulse rate and the blood pressure increased (210 mm Hg) Bilirubin 1 7 mg/100 ml. Electrocardiography revealed sinus tachycardia as the only abnormality Medication with stimulants, penicillin and oxygen produced a slight improvement. Chest X ray showed the same findings as before. On July 10 the patient deteriorated with increasing respiratory difficulties. He was given 2 doses of 20 U corticotropin. In the evening he was mori-



Fig. 3. Photograph of lungs, which are voluminous, dark-red and firm.

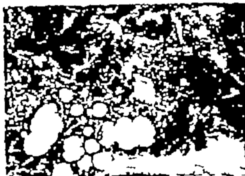


Fig. 4. Picture showing source of intra-alveolar bleeding with moderate fibrous thickening of inter alveolar septa in some areas.

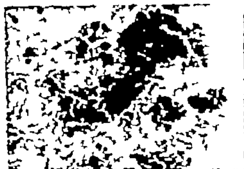


Fig. 5. Sections (preparations stained with Berlin blue and showing massive intracellular and extracellular deposits of iron in the alveoli.

lised with rapid, shallow harsh respiration, and he soon died from respiratory failure.

Post-mortem examination

The body was of ordinary appearance. No signs of jaundice were found. The most striking gross changes were seen in the lungs (fig. 3) which were voluminous and very heavy. The right lung weighed 1 100 g and the left 1,000 g. The lungs were firm and the cut surfaces showed densely crowded rice-seed to hazelnut sized blue-red foci. These lesions were found mainly in the central parts of the lungs. Fresh haemorrhagic foci of varying size were seen in the periphery. The bronchi, which were of normal width with markedly injected mucosa, contained viscous, blood-stained mucus.

The pleural cavities contained no fluid and the pleural surfaces were smooth and shiny. The hilar lymph nodes were the size of hazelnuts and their cut surfaces were of anthracotic appearance. Slightly enlarged lymph nodes of similar appearance were also found around the trachea.

The spleen was moderately enlarged (250 g); it was firm and dark blue-red. The cut surfaces contained distinct follicles.

The liver was also somewhat enlarged (1,800 g). It was brown-red and its anterior edge was rounded. The cut surfaces showed signs of stasis. No gross signs of haemosiderosis were seen.

Microscopical examination

Specimens from the central part of the lung lobes showed large and small alveolar groups containing abundant alveolar phagocytes with engulfed iron pigment (fig. 4). Single inter alveolar connective tissue septa were fibrous and thickened with interstitial deposits of iron. These foci were surrounded by an emphysematous zone. Perivascular accumulations of lymphocytes and leucocytes including eosinophilic lymphocytes were also seen. A moderate accumulation of round cells was also seen in the siderotic foci.

Preparations from the peripheral parts of the lungs showed widespread, intra-alveolar haemorrhagic foci as well as a moderate amount of siderophages (fig. 5). In PAS-stained preparations a moderate amount of

PAS-positive substances were found in alveolar phagocytes as well as interstitially. No iron impregnation of elastic fibres could be demonstrated. Reticulum staining (Foot) showed splitting and fragmentation of argyrophilic fibres in capillary branches of the pulmonary artery.

The findings coincided well with the picture of idiopathic pulmonary haemosiderosis described in acute cases.

Some hilar lymph nodes contained a few small rounded epithelioid cell foci in the marginal zone.

The sinusoids showed a moderate accumulation of shed endothelial cells, some of which showed engulfed iron. No deposits of iron could be demonstrated in the epithelioid cell foci.

Special staining (Berlin-blue) of specimens from the liver, spleen, pancreas and kidneys revelaed no signs of haemosiderosis or of epithelioid cell foci. Neither were any signs of general sarcoidosis observed.

Discussion

The case of fulminant idiopathic pulmonary haemosiderosis is the fourth of its kind on record. Its course appears to have been still more fulminant than the 3 cases published previously (10, 11, 12), the patient having died within about one week of the first serious attack of the disease. In all of the earlier cases the course at least tended to be cyclic with exacerbations and remissions. The fact that slight anaemia was the only laboratory finding in a rapidly fatal disease is also unique.

Whether physical exertion, such as a game of football, can precipitate an attack of pulmonary haemosiderosis is debatable.

Of the examinations performed, chest X-ray showed the most typical signs of idiopathic pulmonary haemosiderosis. The diffuse dissemination of miliary or somewhat larger, partly coalescent parenchymal densities over the entire lung, particularly centrally, is not pathognomonic of pulmonary haemosiderosis. For such findings are also made in miliary tuberculosis, silicosis and other malignant pneumoconioses, pulmonary coniosis in iron and metal workers, pulmonary carcinosis with carcinomatous lymphangitis, actinomycosis, stasis oedema, amyloidosis, miliary bronchitis and peribronchitis in children. But if such a roentgenogram is seen in association with the classical symptoms, shortness of breath, fatigue, pallor and haemoptysis, which are common symptoms, knowledge that such a disease as idiopathic pulmonary haemosiderosis exists might provide the clue to the diagnosis.

Roentgen-examination must, however, probably decide the diagnosis.

In our case the pulmonary changes in the roentgenograms of the chest were relatively moderate compared with the dramatic course of the disease, for in most cases on record the lung changes in the final stage of the disease were much more widespread and massive.

The post mortem findings in acute essential pulmonary haemosiderosis differ from those in the chronic type of the disease.

In our case the findings at autopsy coincided with those described in acute cases (10, 11, 12). In chronic cases the picture is dominated by interstitial pulmonary fibrosis developing as a result of the deposition of iron pigment. In fulminant cases the changes are, of course, fresher. Then the picture is dominated by large intra-alveolar haemorrhagic foci, the interstitial deposition of iron is less marked and no substantial fibrous reactions are seen.

In chronic cases elastic fibres in fibrous, thickened intraalveolar septa and in the

vessel walls are impregnated with iron. It has therefore been supposed that the most important change is that in the elastic system of the lungs, for which haemosiderin is said to have great affinity.

No such change was demonstrated in the acute cases on record. The deposition of the iron in elastic fibres is not a cause but a consequence of the haemorrhages. The change is best comparable to the so-called Gamna-Gandy bodies, which occur in the spleen in portal hypertension and in old infarcts of the spleen.

Pathological investigations of acute cases of idiopathic pulmonary haemosiderosis have revealed that the first stage in the development of the disease is the intraalveolar bleeding. This occurs periodically and produces attacks of dyspnoea and haemoptysis. Haemosiderotic changes in the elastic fibres and pulmonary fibrosis are consequences of the absorption of blood.

Deposits of acid mucopolysaccharides in the elastic fibres in the lungs have been demonstrated by Propst (13) 1955. Similar degenerative changes of the elastic fibres occur in the skin in senile elastosis. Herzog (14) (1954) has demonstrated the occurrence of proliferation and fragmentation of argyrophilic fibrils in capillary branches of the pulmonary artery. It has also been shown, Nanceksevill (15) 1919. Propst (13) 1955, that the severity of the changes in the elastic fibrils varies in proportion to the duration of the disease. No macro- or microscopical changes capable of explaining the periodic intra-alveolar haemorrhages have as yet been observed.

Steiner (16) put forward the theory that the disease is due to an auto-immunization with the lungs as the shock organ. He believes that the disease is due to a local antigen-antibody reaction with consequent changes in the permeability of the pulmonary capillaries. Observations made by Holm (17) 1960 with the gel-diffusion technique according to Ouchterlony also suggest that the disease is due to autoimmunization. He studied haemosiderosis serum for its capacity to precipitate antibodies against normal lung tissue. According to Holm, the results suggest that in pulmonary haemosiderosis there is some sort of auto-immunization possibly an immunological reaction to the patient's own lung tissue.

Halvorsen (7) and others found medication with cortisone to be of therapeutic value.

Splenectomy has often been tried but without any demonstrable effect (Steiner (16) 1954). In all of the published cases the patients had more or less grave anaemia and increased bilirubin values. In their case in a 13-year-old girl Wiesman, Wolvius and Verloop (18) 1953 found a direct positive Coombs test during a crisis, which might suggest a haemolytic component in the disease. In several other cases studied, however, Coombs test has proved negative.

Summary

A case of idiopathic pulmonary haemosiderosis in a 25-year-old man is reported. The case is unique in so far as the patient died from his first severe attack.

The roentgenogram was typical of the disease.

The post-mortem findings coincided with those described in previous early acute cases. The pathological findings in these acute cases of idiopathic pulmonary haemosiderosis reveal that the initial event in the development of the disease

is intra-alveolar haemorrhage. Haemosiderosis changes in elastica and lung fibrosis are consequences of adsorption of the blood.

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From the Medical Clinic (Head Nils Bergqvist, M.D.) Länslasarettet, Ludvika, Sweden

Pure Red Cell Anaemia Successfully Treated with Corticosteroids

Report of a Case

By

Nils Bergqvist

A patient with a peculiar case of anaemia which seems sufficiently interesting to merit publication has been under observation in the Medical Clinic of Ludvika Lasarett since January 1959 i.e. for two and a half years.

The patient, born 1904, an author by profession, has no relevant familial history. He had poliomyelitis aged 6, was operated on for slipped disc in 1946, and underwent subtotal gastric resection (Billroth II) for perforated peptic ulcer in August 1957. There was no postoperative gastric distress, the Hb concentration was 92 per cent six months after the operation.

Having predisposition to dysphoria and headache since early youth, he has for decades and regularly been taking analgesics containing such drugs as phenacetin. Although his intake obviously cannot be precisely estimated, it would appear that in recent years his average daily consumption has constantly been at least 0.6 g phenacetin plus 0.6 g phenazone.

The initial signs of the present disease began around the middle of November 1958 with exhaustion, palpitation of the heart and breathlessness. On December 15th, 1958, he consulted physician who, finding Hb concentration of 61 per cent, prescribed iron tablets. But the patient condition deteriorated and on January 9th, 1959 he was admitted to the Medical Clinic of Ludvika Lasarett.

On arrival the patient was seen to be a well developed male in fairly satisfactory general condition but he was very pale. The rectal temperature was normal. The appearance of the tongue was normal. The superficial lymph nodes were not enlarged and there were no signs of bleeding. The liver and spleen were not palpable. Other findings of the physical examination were non-contributory.

X-ray examinations

Both lungs exhibited minimal, apparently not active apical TB-lesions. The heart was slightly enlarged without any significant configuration. Of the stomach a comparatively small stump remained after subtotal gastric resection a.m. Billroth II. The spleen was not enlarged, the colon, cholecystography and urography were all normal.

Laboratory findings

Blood: Hb 39 RBC 2.0 millions, WBC 3,200 differential unsegmented neutrophils 1 %, segmented neutrophils 39 „ eosinophils 2 basophils 0 lymphocytes 40

Submitted for publication September 13, 1961

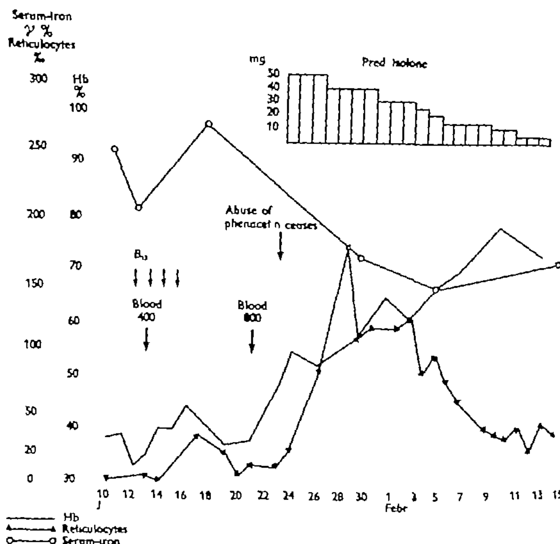


Fig 1 Hb, reticulocyt and serum iron values during the first stay in hospital.

monocytes 18 Platelets 320 000 Reticulocytes 3 ‰ Serum-iron 251 γ ESR 16 mm/hr Bilirubin 0 4 mg Bromsulphalein retention 3.8 GO-transaminase 12 U Serum alkaline phosphatase 1 4 U Thymol turbidity test 1.2 U Serum tamin B_{12} 300 γγ % Serum haptoglobin 112 mg Osmotic resistance haemolysis begins at 0 42 NaCl and is complete at 0 30 NaCl Coombs test negative. NPN 30 mg%

Gastric secretion after histamine stimulation — no free HCl.

Stools-Weber's test negative for blood

Urine-albumin nil or traces. Sediment normal. Sp gr 1 006 to 1 013 after maximal concentration 1 016.

Endogenous creatinine clearance — 146 ml per min.

Bone marrow biopsy

"The specimen is moderately rich in cells and presents a picture where the somewhat immature leukopoiesis is the dominant feature The erythropoiesis is very sparse but of normal type The histological picture deviates completely from that commonly associated with iron deficiency and haemorrhagic anaemia, and there is no evidence of pernicious anaemia. No non-marrow elements are present (Segerdahl)

The proportions of various cellular elements are given in table I

Course (after *January 12th, 1959*)

Hb, reticulocyte and serum iron values recorded during the subsequent course have been plotted in fig 1 where also major therapeutic measures have been inserted.

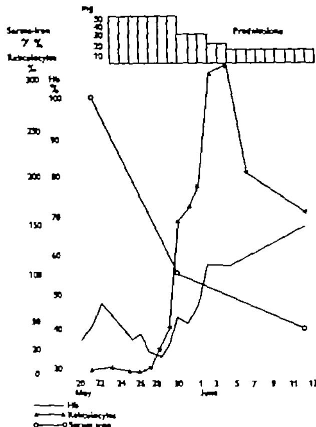


Fig. 2. Hb, reticulocyte and serum iron values during the second stay in hospital.

Although the bone marrow biopsy as well as the normal serum vitamin B_{12} level seemed to rule out megaloblastic anaemia, the patient was given altogether 400 µg vitamin B_{12} from the 12th to 15th of January and on January 15th he received 400 ml of blood. A slight but not significant rise of the reticulocyte count to peak of 37 ‰ ensued. The vitamin B_{12} therapy did not depress the serum iron level. When 800 ml of blood had been administered on January 21st the Hb concentration improved appreciably.

On January 23rd became known that the patient had been taking excessive amounts of phenacetin containing drugs for years and had continued doing so until that every day even whilst in hospital. This abuse of the drug was now stopped and the same day the patient was put on course of prednisolone in an initial daily dosage of 30 mg. The reticulocyte count showed marked improvement on January 26th and attained a maximum of 182 ‰ on January 28th. Concomitantly the Hb concentration began to rise gradually and on February 4th the serum iron level had dropped to 177 γ %. A bone marrow biopsy performed on January 29th disclosed livelier erythropoiesis than on January 12th (cf. table I). Prednisolone treatment was continued at diminishing dosage levels before being discontinued when the patient was discharged on February 14th. The following laboratory findings were made on March 4th, 1959: Hb 80 %, RBC 3.8 million, Reticulocytes 30 ‰, WBC 4,200, Serum iron 190 γ %.

The patient health subsequently remained good until the end of April 1959, when he became ill with nausea and diarrhoea — he himself noticed that the faeces were not unduly dark. The gastrointestinal symptoms ceased, but he felt increasingly fatigued so on May 20th, 1959 he was readmitted to the Medical

Table I Differential counts from five bone marrow biopsies

	12. I 1959	29 I 1959	25 V 1959	6 VI 1959	7 VII 1960
Myeloblasts	0.8	—	0.4	0.8	0.4
Promyelocytes	2.0	0 4	2.8	1.2	2.0
Myelocytes — Neutrophil	26.0	6.0	17.2	8.8	14 0
Myelocytes — Eosinophil	2.0	1.2	1.2	0.4	0.4
Myelocytes — Basophil	—	—	—	—	—
Metamyelocytes	18.0	1.6	10.0	6.4	9.
Leukocytes — Neutrophil — Stabnuclear	12.4	12 4	9.6	6.4	8.8
Leukocytes — Neutrophil — Segmented	12.4	40.8	17.2	4.4	4 4
Leukocytes — Eosinophil	2.4	1.2	1.6	0.4	—
Leukocytes — Basophil.	—	—	0.4	—	—
	76.0	63.6	60 4	8.8	39.2
Proerythroblasts	1.2	1.2	4 4	2.8	0.4
Erythroblasts: Smaller — Orthochromatic	—	—	0.4	2.0	—
Erythroblasts: Smaller — Polychromatic	8.0	16.0	2.0	30 0	18.0
Erythroblasts: Smaller — Basophil	2.0	3.2	0.4	4 4	0.4
Bigger — Orthochromatic	—	—	—	—	—
Bigger — Polychromatic	2.0	1.2	2.8	5.6	11.6
Bigger — Basophil	—	1 6	4 0	4.0	0.4
	13.2	23.2	14 0	48.8	30.8
Monocytes	2.4	2.0	2.8	1.2	1.6
Lymphocytes — Smaller	6.0	11.2	12.4	10.4	18 0
Lymphocytes — Bigger	0.4	—	0.4	0.4	—
Plasma cells	0.8	—	0.8	0.4	1.6
Reticulum cells — Regular	0 4	—	4 0	6.0	8.4
Reticulum cells — Plasmacellular	0.4	—	—	—	6.4
Reticulum cells — Lymphoid	0 4	—	1.6	1.6	—
	10 8	13.2	22.0	20.0	30.0
Atypical	—	—	0.4	—	—
Broken	—	—	3.2	2.4	—

Clinic of Ludvika Lasarett. He assured us that this time he had not taken any drugs containing phenacetin.

This time, too, he was very pale, he exhibited no signs of bleeding and the superficial lymph nodes, liver and spleen were not enlarged

Laboratory findings

Hb 39 RBC 2.3 million. WBC 4,500 differential unsegmented neutrophils 3 segmented neutrophils 56 % eosinophils 1 basophils 1 lymphocytes 27 monocytes 12 Platelets 206,000. Reticulocytes 2 %. Serum iron 284 — Bilirubin 0 7 mg%. Haptoglobin 87 mg% Coombs test negative. NPN 25 mg%

Bone marrow biopsy (May 21st, 1959)

Renewed inhibition of erythropoiesis (cf. table I)

Course

Hb, reticulocyte and serum iron values recorded during the subsequent course will be found in fig 2. This time the only mode of therapy was prednisolone medication whose

Table II. Essential haematological data during the entire period of observation

Date		Hb %	Erythrocytes mill.	Reticulocytes ‰	Serum Iron γ %	Bilirubin mg/100 ml	Leucocytes	Thrombocytes	Dose of prednisolone (mg) in preceding period
1959	10. I	39	2.0	3	231	0.4	5,200	320,000	—
	24. I	56	2.9	24	—	—	11,900	370 000	—
	4. II	67	3.2	98	148	0.4	—	—	See fig 1
	13. II	73	3.5	46	—	—	—	—	
	15 II	—	—	—	—	—	4,600	—	
	4. III	80	3.8	30	190	—	4,200	—	—
	21. V	42	2.4	2	284	0.7	4,500	206,000	—
	30. V	—	—	—	124	—	9,200	—	See fig 2
	6. VI	60	—	208	—	—	—	—	
	12. VI	68	3.4	168	56	—	—	—	
	22. VII	78	3.9	6	195	—	—	—	10
	9 IX	74	3.9	24	—	—	—	—	10
	24. X	66	3.0	22	222	—	—	—	10
	10. XI	75	3.7	41	183	—	—	—	15
1960	13. I	90	4.4	30	183	—	—	—	15
	9. III	84	3.9	15	206	—	—	—	10
	11. V	89	3.9	36	290	—	—	—	10
	16. VIII	81	3.7	26	202	0.4	—	—	10
	12. X	78	3.6	40	175	0.3	—	—	7.5
	7 XII	89	4.0	30	196	0.5	7,800		5
1961	8. II	84	3.6	22	202	—	—	—	2.5
	14. III	82	3.7	15	157	0.5	5,300	203,000	0
	3. V	79	3.6	16	127	—	—	—	0
	23. V	92	4.0	7	148	—	—		0
	16 VIII	82	4.0	16	200	—	—	—	0

effect on the disease picture may be inferred from fig 2. Prednisolone therapy commenced on May 23rd with daily dosage of 50 mg for seven days, whereupon the daily dosage was gradually reduced The reticulocyte count began to rise on M y 28th, peaked at 304 ‰ and 320 on June 2nd and 4th respectively and then declined On May 30th the serum iron level had dropped to 124 from 284 on admission The reticulocyte enhancement was followed by marked and progressive improvement in Hb.

At new bone marrow biopsy (June 6th, 1959) erythropoietic activity was restored (cf table 1

I view of the obvious beneficial action of prednisolone medication and of the relapse into anaemia following the brief prednisolone course given during the first hospitalization period, the prednisolone medication was kept up after the patient discharge on June 6th at daily dosage level of 10 to 15 mg Not until the beginning of August 1960 was it deemed advisable to commence cautious reduction of the dosage The anaemia has not relapsed although reduction of the prednisolone dosage to 10 mg in the autumn of 1959 was attended by slight lowering of the Hb concentration which, however was restored to normal levels when the daily dosage was raised to 15 mg Prednisolone treatment was entirely withdrawn on February 8th,

1961 Table II gives the patient's main haematological data and specifies the prednisolone dosage after the 2nd discharge from hospital.

When the patient was followed up on March 14th 1961 the serum vitamin B_{12} level was 295 γγ% the Shilling test showed a urinary excretion in 48 hours of 14.4 % of the administered dose of radioactive vitamin B_{12}, and the haptoglobin level was 105 mg %. In other words these three tests were all completely normal. As determined by the Cr_{51} method, the erythrocyte survival time was normal (in May 1961)

Renal function tests carried out on March 14th, 1961 showed that the urine was free from albumin the NPN value, was 25 mg. The endogenous creatinine clearance was 84 ml per min. Estimation of renal concentration power yielded a maximal urine specific gravity of 1.013

Results

This case thus occurred in a male patient aged 54 whose stomach had been resected, (Billroth II) 15 months before onset of the illness of interest here. He exhibited no signs of anaemia 6 months after the operation For at least 20 years a constant and excessive intake of phenacetin had taken place, and hyposthenuria indicated mild renal injury but there was no evidence of uraemia After 6 to 8 weeks with progressive symptoms of anaemia severe normochromic anaemia with normal leukocyte and platelet counts supervened. Bone marrow biopsy disclosed hypoplasia of erythroblastoid cells which were not of megaloblastic type. Avitaminosis B_{12} was also contraindicated by the normal serum vitamin B_{12} level. The anaemia was not haemolytic since the bilirubin and haptoglobin levels, the osmotic resistance and Coombs test were normal. The serum iron level was high the reticulocyte count low The patient received some blood transfusions. A series of vitamin B_{12} injections had no obvious effects. The phenacetin abuse was discovered and ceased, and simultaneously prednisolone treatment was instituted This was closely associated with a marked reticulocyte enhancement, falling serum iron levels, Hb concentrations rising towards normal values, and a considerably accelerated erythropoiesis in the bone marrow The prednisolone treatment was withdrawn after 21 days.

Three months after the cessation of prednisolone therapy increasingly severe symptoms of anaemia returned. A grave normochromic anaemia of the same type as before was diagnosed after another 2 to 3 weeks. The erythropoiesis in the bone marrow was once more hypoplastic though not megaloblastic, signs of haemolysis were lacking the serum iron level was high the reticulocyte count very low The patient had not resumed his phenacetin abuse. This time prednisolone therapy was the only form of treatment. Five days after its inception the reticulocyte count started rising and reached a maximum of 320 ‰. The serum iron level dropped in due course the Hb concentration became normal, the erythropoietic structures in the bone marrow proliferated During the subsequent course the daily prednisolone dosage was maintained at 10 to 15 mg After some time this dose was reduced very gradually the drug being withdrawn entirely only after 20 months. The Hb and erythrocyte values remained on the whole normal throughout this time and still remain normal 6 months after the cessation of prednisolone therapy

Discussion

The relevant observations in this case would seem to be that the patient had undergone subtotal gastric resection that he had abused phenacetin for many years,

and that he had two attacks of profound anaemia, the first but not the second preceded by phenacetin abuse. The untreated anaemia both times had the same character-signs of haemorrhage and iron deficiency avitaminosis B_1 and hyper haemolysis were lacking. Bone marrow biopsy demonstrated that inhibition of erythroblastoid elements coincided with low blood reticulocyte counts. These findings are both evidence of reduced erythrocyte production. The high serum iron levels prior to treatment need not necessarily indicate hyperhaemolysis because they might just as well signify a disparity between the demand for and available supplies of iron when haemolysis is normal and erythrocyte and haemoglobin production are reduced.

Both the severe attacks of anaemia were followed by a rapid remission characterized by substantial increase of bone marrow erythroblastoid elements, a sharp rise in the blood reticulocyte count, and return to normal of the excessive serum iron level. Even if vitamin B_1 injections, some blood transfusions and cessation of long-established phenacetin abuse somewhat obscure the picture it is highly probable that the first remission was a response to prednisolone medication. As regards the second remission, when prednisolone therapy was the only mode of treatment the relationship between therapy and response is too obvious to leave room for doubt.

After the patient first remission, the prednisolone treatment was discontinued on February 14th, 1959 Some weeks after onset of rapidly exacerbating symptoms of anaemia severe anaemia was diagnosed anew on May 20th, 1959 i.e. when be 100 days had lapsed, a period of the same order as the normal survival time of erythrocytes. This suggests that the new erythrocytes released into the blood during the first remission underwent to erythrocytolysis more or less *en masse* without having their ranks filled owing to inhibition of the bone marrow's erythrocyte production. Probably the great prolongation and the gradual withdrawal of the prednisolone medication during the second remission has protected the patient against further relapses of anaemia. His Hb concentration and erythrocyte values were still normal 6 months after complete cessation of prednisolone therapy

The form of anaemia displayed by this patient is patently extremely rare. No similar case has been reported in the literature available to the author Some of the features of this case resemble Gasser's (1) 10 cases of Akute Erythroblastopenie in children In these cases the erythrocytes temporarily disappeared from the bone marrow where pathological giant cells resembling proerythroblasts were observed. Gasser attributed the phenomenon to toxic or infectious factors.

The present case in addition had some similarity to those aplastic erythropoietic crises which Owren (2) has described in haemolytic spherocytic anaemias. Clearly however this was not such a case of haemolytic anaemia since signs of hyper haemolysis were absent during both the acute attacks and the subsequent observation period. Estimation of the CO content of expiratory air – the most sensitive criterion of hyperhaemolysis — was not available to us. The comparatively high serum iron levels and reticulocyte counts noted from time to time during the long observation period after the second hospitalization would *per se* justify a suspicion of hyperhaemolysis were it not for the contrary evidence furnished by the haptoglobin level (March 14th, 1961) and the

normal erythrocyte survival time estimated by the Cr_{51} method (May 1961)

A case of combined haemolytic anaemia and bone marrow injury reported by Bonham-Carter et al. (3) is not without interest in this connection mainly owing to the therapeutic efficacy of steroid medication In a boy of 6 who recently had a haemolytic crisis with positive Coombs test a catarrhal infection was attended by recrudescence of severe anaemia This time the blood lacked reticulocytes and the bone marrow exhibited only 1 5 % erythroblasts. Cortisone and ACTH medication at the same time as splenectomy gave rise to a temporary improvement of the anaemic state. Cessation of steroid therapy was followed by exacerbation of the haemolytic anaemia, reticulocytes and erythroblasts vanished entirely erythrocytolysis accelerated Massive steroid therapy was accompanied by an elevation of the bone marrow erythroblast content to 68 % and of the blood reticulocytes to 75 ‰ and by Coombs test becoming negative. Bonham Carter et al. surmised that the anti-erythrocyte antibodies shown to be present in this case had destroyed not only erythrocytes and reticulocytes but also bone marrow erythroblasts.

The fact that our patient had undergone gastric resection was probably not pathognomonic for the anaemia. Gastric resection may give rise to iron deficiency and avitaminosis B_1 and these in turn to anaemia but in the present case there were no signs of either of these deficiency states.

It now remains to consider the question as to whether the patient's phenacetin abuse might have been a factor in the causation of his anaemia. Excessive phenacetin intake had indubitably occurred, and the observed signs of moderate renal damage were in good agreement with the symptoms of phenacetin induced interstitial nephritis. However the anaemia associated with phenacetin abuse is haemolytic in nature (4) which as mentioned the present case of anaemia was not. During the initial stage of the disease, when phenacetin abuse still was going on, no attempts were made to demonstrate the presence in the erythrocytes of the so-called Heinz Innenkörperchen (5) seen in phenacetin induced anaemias (4) Nor was the blood examined spectroscopically

It seems obvious that our patient did not have phenacetin anaemia of the usual haemolytic type but that his erythropoiesis was inactivated by aplastic or at least hypoplastic crises. The beneficial effects of steroid therapy suggests that some kind of antigen-antibody mechanism might be the factor responsible for erythropoietic inhibition or perhaps destruction of erythroblastoid cellular elements. Unfortunately nothing is known about the nature of these presumed antigens and antibodies. Whether the phenacetin was involved in their production is merely a matter for idle speculation. The persisting benefits of the prolonged steroid treatment kindles the hope that this hypothetical immunological mechanism may have been put out of action.

Summary

A case is reported of severe normochromic anaemia in a male of 54 who had previously undergone subtotal gastric resection and for decades constantly had been taking excessive amounts of phenacetin containing drugs and exhibited signs of mild interstitial nephritis unaccompanied by uraemia. Bone marrow biopsy disclosed erythroblastic hypoplasia, and low blood reticulocyte counts

also suggested diminished erythropoiesis, whereas signs of iron deficiency avitaminosis B_{12} and hyperhaemolysis were lacking. A comparatively short period of prednisolone therapy was probably responsible for a rapid remission. Three or four months later the patient sustained a severe relapse of the same type of anaemia. On this occasion, too, prednisolone treatment was attended by a prompt and complete remission. During a continuation for 20 months of the prednisolone therapy at low and very slowly diminishing dosage levels the blood values remained normal and six months after the final withdrawal of therapy they were still normal. The nature of the anaemia and part played by phenacetin in its induction are discussed.

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normal erythrocyte survival time estimated by the Cr_{51} method (May 1961).

A case of combined haemolytic anaemia and bone marrow injury reported by Bonham Carter et al. (3) is not without interest in this connection, mainly owing to the therapeutic efficacy of steroid medication. In a boy of 6 who recently had a haemolytic crisis with positive Coombs test a catarrhal infection was attended by recrudescence of severe anaemia. This time the blood lacked reticulocytes and the bone marrow exhibited only 1.5 % erythroblasts. Cortisone and ACTH medication at the same time as splenectomy gave rise to a temporary improvement of the anaemic state. Cessation of steroid therapy was followed by exacerbation of the haemolytic anaemia, reticulocytes and erythroblasts vanished entirely, erythrocytolysis accelerated. Massive steroid therapy was accompanied by an elevation of the bone marrow erythroblast content to 68 % and of the blood reticulocytes to 75 ‰ and by Coombs test becoming negative. Bonham-Carter et al. surmised that the anti-erythrocyte antibodies shown to be present in this case had destroyed not only erythrocytes and reticulocytes but also bone marrow erythroblasts.

The fact that our patient had undergone gastric resection was probably not pathognomonic for the anaemia. Gastric resection may give rise to iron deficiency and avitaminosis B_1 and these in turn to anaemia, but in the present case there were no signs of either of these deficiency states.

It now remains to consider the question as to whether the patient's phenacetin abuse might have been a factor in the causation of his anaemia. Excessive phenacetin intake had indubitably occurred and the observed signs of moderate renal damage were in good agreement with the symptoms of phenacetin induced interstitial nephritis. However, the anaemia associated with phenacetin abuse is haemolytic in nature (4) which, as mentioned, the present case of anaemia was not. During the initial stage of the disease, when phenacetin abuse still was going on, no attempts were made to demonstrate the presence in the erythrocytes of the so-called Heinz Innenkörperchen (5) seen in phenacetin induced anaemias (4). Nor was the blood examined spectroscopically.

It seems obvious that our patient did not have phenacetin anaemia of the usual haemolytic type but that his erythropoiesis was inactivated by aplastic or, at least, hypoplastic crises. The beneficial effects of steroid therapy suggests that some kind of antigen-antibody mechanism might be the factor responsible for erythropoietic inhibition or perhaps destruction of erythroblastoid cellular elements. Unfortunately nothing is known about the nature of these presumed antigens and antibodies. Whether the phenacetin was involved in their production is merely a matter for idle speculation. The persisting benefits of the prolonged steroid treatment kindles the hope that this hypothetical immunological mechanism may have been put out of action.

Summary

A case is reported of severe normochromic anaemia in a male of 54 who had previously undergone subtotal gastric resection and for decades constantly had been taking excessive amounts of phenacetin containing drugs and exhibited signs of mild interstitial nephritis unaccompanied by uraemia. Bone marrow biopsy disclosed erythroblastic hypoplasia, and low blood reticulocyte counts

also suggested diminished erythropoiesis, whereas sgns of iron deficiency avitaminosis B_{12} and hyperhaemolysis were lacking. A comparatively short period of prednisolone therapy was probably responsible for a rapid remission. Three or four months later the patient sustained a severe relapse of the same type of anaemia. On this occasion, too prednisolone treatment was attended by a prompt and complet remission. During a continuation for 20 months of the prednisolone therapy at low and very slowly diminishing dosage levels the blood values remained normal and six months after the final withdrawal of therapy they were still normal. The nature of the anaemia and part played by phenacetin in its induction are discussed.

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Corticotropin in the Treatment of Infectious Mononucleosis

By

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There is no specific treatment for infectious mononucleosis. Attempts ha e been made to treat the disease with many different kinds of drugs, among others antibiotics. In recent years, however a number of papers ha e been published reporting that corticotropin and corticosteroids have a favourable effect on the course of the disease (1 2, 3 4 5 6, 7 8) All of these publications, however are based on single cases or small series without a control series from the same period. The course of infectious mononucleosis is unpredictable and very variable, and w therefore feel there is a need to study the effect of corticotropin in infectious mononucleosis using a larger material with simultaneous controls.

Material and methods

The presen study was carried out in the Aurora Hospital in the period December 1 1956—March 31 1961 The patients with mononucleosis admitted to the hospital on the odd days of each month received during five days corticotropin intramuscularly and potassium salt of V-penicillin (V Pen forte, Orion) orally Patients admitted on even dates were given penicillin only for the same length of time The dosage of corticotropi for adults and children over 10 years of age was as follows First day of treatment, 60 I U of long-acting corticotropin (Cortrophin-Z, Organon) second day 40 I. U third and fourth days, 20 I U each and fifth day 10 I U One-half the dosage was used for children under 10 but the time of treatment was the same.

In this study were included only those patients with fever and exudative tonsillitis t the time of commencement of treatment, since the duration of fever and tonsillar exudates is an important criterion in the evaluation of the effect of treatment. Since Paul-Bunnell negative mononucleosis is special problem, only those patients were selected for study who had a titre of 1 64 or more.

Our material consisted of 111 patients whose diagnosis of infectious mononucleosis was made on the basis of the clinical, haematological and serological findings. Ther were 54 males and 57 females 26 were aged under 15 and 85 were over 15. Of these 51 patients wer treated with corticotropin (corticotropin group) and 60 patients were used as controls (control group).

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Table I Duration of fever and tonsillar exudates in patients treated with corticotropin and in the control series

Duration of fever before treatment (days)	Number of patients		Mean total duration of fever		Mean duration of fever after treatment begun		Mean duration of exudate after treatment begun	
	Cortr group (51)	Control group (60)	Cortr group	Control group	Cortr group	Control group	Cortr group	Control group
0–2	8	18	2.5	5.4	1.8	4.3	5.3	5.8
			p <	0.001	p <	0.01	p =	
3–4	8	15	5.1	8.1	1.5	4.6	5.4	6.0
			p <	0.001	p <	0.001	p =	
5–7	14	13	9.5	9.6	3.9	3.8	6.9	6.2
			p =		p =		p =	
8–	21	14	13.8	16.2	2.6	3.8	6.7	4.9
			p =		p =		p =	

The treatment was begun on 102 patients as soon as they were admitted, since a diagnosis of mononucleosis appeared probable on the basis of the clinical picture and the haematologic findings. This rapid diagnosis was possible because during the period of this study a blood examination was performed immediately on admission in all cases of tonsillitis or of suspected mononucleosis. In 9 cases the treatment was begun 1—6 days after admission, since the diagnosis of mononucleosis was not reached earlier. However in the latter cases too the choice of treatment was determined by the date of admission.

The routine before commencement of treatment included a complete blood examination, Paul Bunnell test, cultures fo haemolytic streptococci from nasal and pharyngeal swabs, total serum bilirubin determination, and thymol turbidity test. The blood examination, Paul Bunnell test and haemolytic streptococci determinations were repeated on all patients five days after treatment was begun and then at intervals of one week in the hospital. The blood count and the Paul Bunnell titre continued to be observed at follow-up examinations after discharge from hospital. Electrocardiograms and roentgenography of the chest were made in all cases. Changes in the clinical condition were recorded in the case report daily.

Results

The emphasis in this study was on the duration of fever and tonsillar exudate. The patients were distributed into four groups according to the duration of fever before commencement of the treatment.

As is seen in table I the mean total duration of the fever and the duration since the beginning of treatment were clearly shorter in the group given corticotropin than in the control series if the fever before the commencement of treatment had lasted for four days or less. The difference is statistically significant (P = 0.001). One of the patients given corticotropin had a rise of fever immediately after discontinuation of corticotropin administration (relapse) but the temperature reverted to normal and remained so when the corticotropin therapy was resumed.

It is clearly seen from the table that the corticotropin therapy had no effect on the duration of the tonsillar exudate and on the course of the tonsillitis as judged by the exudate. The number of leukocytes

and of atypical lymphocytes was also found not to be influenced by corticotropin. The same was true of the Paul-Bunnell reaction.

In the absence of objective means of examination it is more difficult to evalu ate the patient s general condition, subjec tive distress, and the enlargement of the lymph nodes. The impression was gained in many cases that corticotropin promoted the improvement of the patient s general physical condition and abatement of the distress. In some cases such an effect was not observable. The same was true of regression of the enlarged lymph nodes.

Discussion

Treatment with corticotropin and cor ticosteroids has been attempted, with varying success, in many viral diseases, to which infectious mononucleosis proba bly also belongs (10) Except in infectious hepatitis, on which these drugs often have an indisputable effect, the results have been negative or contradictory and call for further investigation. Thus, when a simultaneous control series was used, corticotropin and cortisone were found to have no effect on mumps orchitis (11 12) although an effect has been claimed by many authors. The egression of lymphatic hyperplasia and the lymphopenia following the administration of these drugs are incontestable facts in general pathology It is therefore only natural that at tempts hav been made to employ corticotropin and corticosteroids in the treat ment of his infectious mononucleosis, sinc this disease is characterised by severe inflammation and reaction of the lymphoid tissue W are not aware, however of studies in which a simultane ous control series was used.

If the treatment was begun at an early stage of the disease, defervescence was statistically significantly more rapid in our series of 51 patients treated with corticotropin than in the control series of 60 patients. That corticotropin shortens the duration of fever in patients with infectious mononucleosis is in agreement with the findings of other workers (1 3 4 6) It is difficult to say whether the shorter duration of fever is due solely to the non-specific antipyretic effect of corticotropin (13)

Contrary to what other investigators (3 6) have reported, we were unable to observe in our series that the disappear ance of the tonsillar exudate nd healing of the tonsillitis were promoted by corticotropin. We also failed to find a definite difference between the corticotropin group and the control group with respect to regression of the lymph node enlarge ment. However estimation of the size of the lymph nodes is difficult in the absence of exact methods of measurement. It also is very difficult to estimate the abatement of subjective sensations of pain and the improvement of well-being The impression gained by us in many cases that corticotropin reduces the toxicity of infectious mononucleosis is n greement with observations made by other investigators (1 3 4) The euphoristic and possibly analgesic effects of corticotropin (6) may be factors in these favourable symptomatic effects, which further may be enhanced by the shorter duration of fever

According to reports in the literature, corticotropin and corticosteroids have been used chiefly in the treatment of those patients with mononucleosis who have severe, typical forms of the disease, and especially if there are certain complications However in the light of the

present study the use of corticotropin is usually not worth while in the treatment of infectious mononucleosis. Nevertheless, in highly febrile toxic cases it may prove beneficial by giving symptomatic relief. Its administration is indicated in certain rare complications of mononucleosis such as thrombocytopenic purpura and acquired haemolytic anaemia

Summary

In 51 cases of infectious mononucleosis the treatment was corticotropin intramuscularly and penicillin orally during five days. At the same time a control series of 60 patients was given penicillin alone.

The corticotropin therapy was found to shorten the duration of fever The difference from the control series was statistically significant if there had not been fever for over four days before the commencement of the treatment. On the other hand, the corticotropin treatment was not found to have an effect on the duration of tonsillitis, using the duration of tonsillar exudate as criterion, on the number of leukocytes and atypical lymphocytes or on the Paul Bunnell reaction. The impression was gained that the corticotropin in some cases promoted improvement of the general condition and abatement of the subjective distress but in view of the rather subjective nature of these symptoms, no definite conclusions can be drawn in this respect from the present study The same remark applies to the severity and duration of lymphadenopathy Corticotropin is therefore not recommended in the treatment of infectious mononucleosis, except that in certain severe cases of the anginoseglandular form attended with high fever it may be recommended in combination with antibiotics. Corticotropin is also indicated in some unusual complications of the disease.

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Ornithosis Pneumonia with Special Reference to Roentgenological Lung Findings

By

R. Stenström, E. Jansson and O Wager

The progress of therapy for pulmonary diseases has made the accurate knowledge of their etiology increasingly important. Nowadays acute ornithosis pneumonia also must be considered in differential diagnosis.

In the classical roentgenological view cuneiform infiltrates are demonstrabl in connection with ornithosis pneumonia. They spread hilifugally chiefly into the basal portions of the lungs (6, 11 16) Maué (10) stated that th cuneate pulmonary changes are not specific for ornithosis but are present also in other pneumonias Of more recent workers reporting on ornithosis pneumonia mention might be mad of Rejnberg et al. (12) Borch Jorgensen et al. (2) Glawatz et al. (3) and Gordon (4) They all came to the conclusion that tru ornithosis pneumonia resembles primary atypical pneumonia. Earlier the pneumonia caused by this disease had been regarded as bronchopneumonia.

A summary was compiled by Lillie (8) of the anatomical changes on the basis of 9 cases of his own and 43 cases published in the literature. The interstitial tissue is oedematous, and extensive cellular infiltrates, lymphocytes, macrophages, mast cells and sometimes also granulocytes are established in a half of the cases. When the proliferative tissue reaction increases and the interstitial tissue becomes denser the air content of the lungs diminishes. The alveoli are partly over-enlarged, partly dystelectatic There are moreover often alveolar islets containing serum, red cells, macrophages and sometimes even some fibrin. Guthert (5) stated that numerous alveolar cells are seen in the alveoli in a certain phase of ornithosis pneumonia. He consequently called ornithosis pneumonia from the histological

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Table I. Age distribution of patients with ornithosis

Age, yrs.	1—5	6—10	11—15	16—20	21—30	31—40	41—50	51—60	> 60	Total
Number	3	6	2	2	3	5	4	4	—	29

standpoint "alveolar cell pneumonia" and considered this finding specific for the etiology in question.

The conclusion arrived at by Rejnberg et al. (12) and Glawatz et al. (3) on the basis of these anatomical changes was that the condition in question is principally an interstitial pulmonary disease associated in varying degrees with transudate and focal alveolar processes. In general the disease is initially a true interstitial disease in which the roentgenogram displays only a thin veil-like or hyaline more or less homogeneous infiltrate. This infiltrate is not dense; on the contrary, the reticular structure is readily distinguishable and likewise the vascular shadows and peribronchial bands. The transparency is attributable to the fact that the air content of the alveoli is virtually undiminished. Rejnberg and Glawatz regard the transparency as the equivalent of an extra-alveolar process. The picture often changes later on because exudate forms in the alveoli but even then thinner, transparent, generally reticular areas are seen in the marginal area of the changes. The cuneiform areas mentioned earlier are according to Rejnberg and Glawatz simply atelectases. Roentgenological changes can accordingly be dependent on the phase of the disease in which the roentgenogram was made. A pneumonia that perhaps appears initially to be atypical may in a later phase resemble more bronchopneumonia or lobar (segment) pneumonia.

Numerous cases of ornithosis pneu- [illegible] were established in a study of the incidence of ornithosis infection in Helsinki. The principal results have already been published (7).

Method

The series consisted of 29 cases of ornithosis pneumonia treated at Aurora Hospital, Helsinki between October 8, 1958, and March 15, 1960.

This series was obtained by performing systematic ornithosis antibody studies on 559 pneumonia patients under treatment at the hospital during this period. Two serum samples were taken from all these patients at an interval of 10—14 days. If a high ornithosis antibody titre ($\geq$ 1:40) was established, two more serum samples were examined afterwards.

The ornithosis antibody study was carried out at the Bacteriological Laboratory of Aurora Hospital. The technique employed has been described elsewhere (7). Ornithose antigen of Behringwerke was used as the antigen. The highest serum dilution (before adding the reagents) which gave at least 75 per cent complement fixation was taken as the titre.

The criterion for the classification of a case of pneumonia as acute ornithosis was that the serological examination of 2 or more serum samples from the patient met one or several of the following conditions:

1) a definite rise in the ornithosis antibody titre during the illness to a high level, followed by a fall;

2) a 4-fold or greater rise in the antibody titre during the acute phase, or an at least 4-fold drop in titre during recovery;

3) an ornithosis antibody titre of such a high level that it was considered to have been caused by the acute disease. Titre 1:60 was selected as the borderline.

A further requirement was that the patient's serum gave a negative result against the control antigen.

The 29 patients of the ornithosis series accounted for 5 per cent of the pneumonias treated at the hospital during the period. These cases were distributed as follows according to the criteria that they fulfilled 10 belonged to group 1), 14 to group 2) and 5 to group 3)

Fifteen of the patients were women and 14 men. The age distribution is given in table I There were 11 children. Information on possible contact with birds was obtained from 27 patients. Ten of them reported close contact with pigeons. The incidence of the disease was highest in the autumn.

The ornithosis antibody titre generally reached its peak within the first 3 weeks of the disease. When 5—17 months had elapsed from the acute phase of the disease, antibodies were no longer demonstrable in 5 patients and 8 patients gave an antibody titre of 1 10.

Clinical picture

A more detailed analysis of the clinical picture is given elsewhere (7) It was rather variable. Several patients, however had severe dry cough which persisted for weeks High fever was another typical feature although few patients had only slight rise in temperature.

The erythrocyte sedimentation rate (ESR) was fairly high in many patients, especially if they had been ill at home for some time without adequate antibiotic treatment. The white cell count was generally no higher than normal in fact it was lowered in some cases. Lues reactions were examined in 27 patients five of them (26 per cent) gave positive Wassermann reaction, Wassermann cholesterol reaction and/or Kahn. Sitolipin was negative in all the patients The Wassermann reaction remained positive for as long as two months

Results of the roentgenological studies

The series is distributed as follows according to localization of the pneumonic foci right lung 10, left lung 12, bilateral 7 cases The distribution of the foci by lobes was as follows

	cases
Right inferior lobe	4
Left inferior lobe	5
Right superior lobe	2
Left superior lobe	4
Various combinations of the lobes of the right lung	3
Various combinations of the lobes of the left lung	3
Combinations of both inferior lobes	6
Other combinations of both lungs	2
	29

Distribution of the series according to Schmid and Weber (14) shows that group A, i. e. segmental lobar pneumonias, comprised only 2 cases and that all the other 27 cases belonged to the heterogeneous group B Fourteen of these cases were primarily atypical pneumonias, 10 bronchopneumonias and 3 intermediate forms which were difficult to classify Group A, i. e. the group of segmental lobar pneumonias, included cases with homogeneous infiltrates in which no signs of atelectasis were seen and which were restricted to a segment or lobe. In the atypical pneumonia group the infiltrates were band-like and/or reticular transparent, hilifugal and fan-shaped.

The nature of the dominating roentgenological shadows was

	cases
Homogeneous shadows	11
Spot-like shadows	7
Band-like shadow	11
	29

Spot-like infiltrates preponderated in infiltrates of the bronchopneumonia type.

Atelectases were established in 10 cases, i. e. about a third of the series. Signs of pleural reaction were demonstrable in 16 patients. Both cases of segmental pneumonia showed pleural reaction, but the distribution was uniform in the other groups. Hyperplasia of the hilar glands

Table II Patients with ornithosis distributed according to duration of roentgenologic changes

Duration of roentgenologic changes	No. of patients
< 3 weeks	2
3—6 weeks	13
6—9 weeks	9
> 9 weeks	5
Total	29

was established in two-thirds of the cases, 19 patients. A pulmonary abscess devel oped in 1 case (no 9)

Table II shows how long after the onset of the disease the roentgenological changes persisted They often took a long time to disappear

Case reports

The following cases were selected to il lustrate the variability of the roentgenological finding in ornithosis pneumonias. According to the serological criteria 2 cases (3 and 9) belonged to group 1) and 3 (20, 21 and 24) to group 2) WaR was positive in 2 cases (20 and 21)

Case 3 Male, a pigeon exterminator aged 49 On February 14 1959 the temperature rose to 40 C and there was pain in limbs, slight headache and the eyes were somewhat sensitive to light. He was admitted on the same day

A lung roentgenogram on February 17 revealed in the area of the posterobasal seg ment of the right lower lobe a reticular band like infiltrate which was broadly connected to the hilum Small spots even were seen at the points of contact of the bands (fig 1) The change was clearly transparent there was also slight pleural reaction and somewhat enlarged hilum. The changes had decreased in the roentgenograms taken on March 9 and March 23 On April 4 the finding was normal

Case 20 A boy aged 7 became ill with a cough and fever in early September 1959 Lung roentgenograms taken on September 16 and October 6 showed in the area of the lower and middle lobe of the right lung a band like transparent pattern and, especially in the region of the middle lobe, a reticular pattern and small radiotranslucent areas (fig 2) A minimal pleural reaction and some ate lectasis were also seen. The roentgenogram taken on October 14 displayed only hyper plasia of the hilar glands, no infiltrate

Case 21 A woman aged 27 In the summer of 1959 she had had a cough with a slight rise in temperature. At the end of September the cough was worse and the evening tem perature was c. 38° C.

A lung roentgenogram on October 22 revealed patchy faint infiltration in the area of both upper lobes, especially in the region of their posterior segments (fig 3) The hilar pattern was also profuse. The lesion was roentgenologically very suspiciously like TB, and the patient was admitted to hospital. On November 7 however the roentgeno logical changes had disappeared almost com pletely

Case 24 A man aged 35 His illness began on October 11 1959 with a chill followed shortly by a cough and hoarseness. On Octo ber 24 the temperature was 38 C and there was pain in the chest.

A lung roentgenogram taken on October 21 revealed a homogeneous, poorly trans parent infiltrate in the left lingula. There was no atelectasis in the infiltrate but it showed a distinct pleural reaction and hyperplasia of the hilar glands. The X-ray finding was essen tially unchanged on October 27 (fig 4). A reticular band-like structure was seen at the margin of the translucent area. It resembled the typical changes present in ornithosis. The possibility of TB and tumour was considered.

On tomography the lesion appeared, how ever to be inflammatory and thus countered th suspicion of tumour Only remnants of the changes were seen after 7 1/2 weeks and after 2 1/2 months they had almost disap peared

Case 9 A man aged 52. On December 27 1959, he became ill with a cough, and fever of 39 6 C. He complained of pain on the left side and the sputum was slightly blood-stained He was admitted on December 29 with the diagnosis Pleurisy?

A lung roentgenogram taken on January 4 1960 revealed a homogeneous infiltrat n the



Fig 1 Case 3 In the area of the right lower lobe is transparent, reticular uniform infiltrate, spreading from the hilum toward the periphery Roentgenologically atypical pneumonia



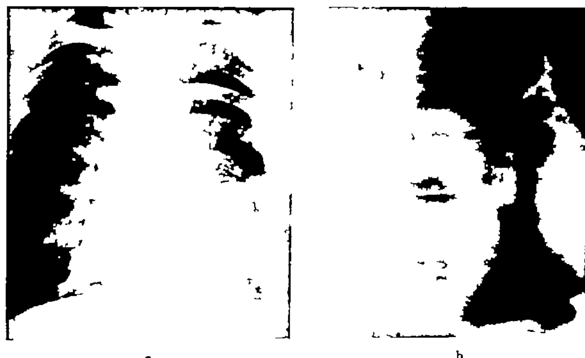
Fig 2. Case 20. In the area of the middle lobe and the lower lobe can be seen reticular pattern, small radiotranslucent areas, minimal pleural reaction and atelectasis, and incipient hyperplasia of the hilar glands. Roentgenologically atypical pneumonia.



Fig 3 Case 21 There is patchy faint infiltrate and also profuse hilar patterning in the area of both upper lobes, especially the posterior segments. The changes were first suspected as TB roentgenologically but they disappeared completely in 2 weeks



Fig 4 Case 24 Poorly transparent infiltrate localised chiefly in the lingula segment. The infiltrate reveals no atelectasis but shows pleural reaction and hyperplasia of the hilar glands At the cranial margin the reticular structure is typical of ornithosis pneumonia. The change was also suspected to be TB and tumour



a b

Figs. 5 a and b. Case 9 There is a homogeneous, dense infiltrate and fluid level, and ca ty in the left lower lobe. Pleural reaction and hyperplasia of the hilar glands are also demonstrable. Tomogram 5 b shows a cavity with irregular margins which is a suspected tumour The change decreased after 6 months and was regarded as the sequela of an inflammation.

area of the apical and posterobasal segments of the left lower lobe and pleural fluid in the left costophrenic sinus. Glandular hyperplasia was established in the left hilum. A roentgenogram taken on January 11 revealed a cavity formation and fluid level (fig 5 a) and tomograms (fig 5 b) on January 15 showed an irregular cavity with which the bronchus seemed to have no communication. The finding was suspected tumour On March 15 the roentgenogram showed that the lesion had become denser and more clearly demarcated tumour was still suspected. Bronchoscopy failed, however to show anything indicative of tumour Six months from the onset of the disease the change had decreased and was regarded as the sequela of an inflammatory process. It had disappeared in the roentgenogram taken on December 12 1960

Discussion

It is common knowledge that the frequency of pneumonia in children is highest in infants and children of pre school age. This was true also of the pneumonia series of 893 cases from Aurora Hospital (1956 15) In the present work the highest incidence of ornithosis pneumonia coincides with the school-going age.

The localisation of the pneumonic foci showed no preponderance in the right lung The frequency of various lobe combinations, on the other hand was fairly high Segmental pneumonias were rare. About a half of the cases resembled atypical pneumonia and this form of the disease was thus c. 10 times more frequent than in the series published in 1956 in which the incidence of atypical pneumonias was 5 per cent.

Atelectases were roughly as numerous as in the 1956 series, i e 34 per cent (1956 29 per cent) Pleural reaction, on the other hand was clearly more common

in the present series, i 55 per cent (1956 15 per cent) Pleural reaction thus does not appear to be rare, as Schinz (13) claimed, and the present results concur well with the view put forward by Glawatz (3) The same applies to hilar hyperplasia which was established in 66 per cent of the cases. The authors can thus agree with Glawatz that pleural reaction and hyperplasia of the hilar gland may assist in differential diagnosis.

The present workers can also concur with the view advanced by Rejnberg (12) and Glawatz (3) that the pulmonary structure is reticular of hyaline transparency in ornithosis pneumonia. This appears to be a fairly typical feature of ornithosis pneumonia. This is obviously true only in pure cases and not in complicated, although the original reticular structure is visible even in the latter at the margins of the infiltrate.

The changes in ornithosis pneumonia persist for relatively long, according to Rejnberg (12) Especially the reticular forms, he states, last c. 6—7 weeks. In the present series, too almost half of the pneumonias lasted for over 6 weeks. Glawatz (3) on the other hand, said that ornithosis pneumonias rarely last more than 3 weeks.

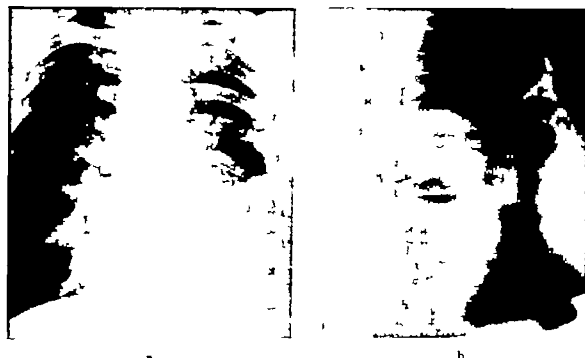
A tumour was suspected once and tuberculosis 4 times in the present series. An abscess developed in the case with a suspected tumour One tumour suspect with ornithosis pneumonia was reported by Borch Jörgensen (2) Tarnowski (17) found 3 cases of ornithosis pneumonia among the 100 on record in tuberculosis district offices. Anderson (1) and Mathiesen (9) mentioned the significance of tuberculosis and neoplasm for differential diagnosis They are in fact important points in differential diagnosis. It is advisable in obscure cases to perform ornithosis antibody studies in order to arrive at the correct diagnosis. The same naturally applies also to Wassermann positive lung infiltrates.

Summary

Of the 539 patients with pneumonia treated at Aurora Hospital over a period of 1 1/2 years and studied serologically for a possible ornithosis etiology 29 (5 per cent) fulfilled the criteria stated for ornithosis pneumonia Eleven of these 29 cases were under 16 years of age. The frequency among children was highest in those of school age. Besides prolonged, severe dry cough, high fever and often a high erythrocyte sedimentation rate, 26 per cent of the patients gave positive WaR and/or Kahn reaction.

Lung X-rays revealed roentgenological changes reminiscent of atypical pneumonia in half the patients. The changes were typically bandlike, reticular and transparent infiltrates. They were hilifugal and fan-shaped. Most of the cases differing from this typical finding displayed a transparent, reticular pattern in the marginal areas of the infiltrates. Pleural reaction and hyperplasia of the hilar glands were common in the present series. The roentgenological changes caused by ornithosis generally persisted for a long time, over 6 weeks in 48 per cent and more than 9 weeks in 17 per cent. On the basis of the roentgenological finding tuberculosis initially was suspected in 4 patients and a tumour in 1 patient. An abscess later developed in the case of suspected tumour

Ornithosis antibody study is advisable in all cases of pulmonary infiltrates of uncertain etiology and in pneumonia cases with positive lues reactions.



a b

Figs. 5 a and b. Case 9 There is a homogeneous, dense infiltrate and fluid level, and cavity in the left lower lobe. Pleural reaction and hyperplasia of the hilar glands are also demonstrable. Tomogram 5 b shows a cavity with irregular margins which is a suspected tumour The change decreased after 6 months and was regarded as the sequela of an inflammation.

area of the apical and posterobasal segments of the left lower lobe and pleural fluid in the left costophrenic sinus. Glandular hyperplasia was established in the left hilum. A roentgenogram taken on January 11 revealed a cavity formation and fluid level (fig 5 a) and tomograms (fig 5 b) on January 15 showed an irregular cavity with which the bronchus seemed to have no communication. The finding was suspected tumour On March 15 the roentgenogram showed that the lesion had become denser and more clearly demarcated tumour was still suspected. Bronchoscopy failed, however to show anything indicative of tumour Six months from the onset of the disease the change had decreased and was regarded as the sequela of an inflammatory process. It had disappeared in the roentgenogram taken on December 12, 1960.

Discussion

It is common knowledge that the frequency of pneumonia in children is highest in infants and children of pre school age. This was true also of the pneumonia series of 893 cases from Aurora Hospital (1956 15) In the present work the highest incidence of ornithosis pneumonia coincides with the school-going age.

The localisation of the pneumonic foci showed no preponderance in the right lung The frequency of various lobe combinations on the other hand was fairly high. Segmental pneumonias were rare. About a half of the cases resembled atypical pneumonia and this form of the disease was thus c. 10 times more frequent than in the series published in 1956 in which the incidence of atypical pneumonias was 5 per cent.

Atelectases were roughly as numerous as in the 1956 series, i e 34 per cent (1956 29 per cent) Pleural reaction, on the other hand was clearly more common

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Red Cell Triiodothyronine Uptake in Relation to the Result of Treatment in Thyrotoxicosis and Myxedema

By

H. Doorenbos, A. Bakker and M. G. Woldring

Recently the red blood cell triiodothyronine uptake (the so-called red cell T3 uptake) has been added to the methods for assessing thyroid function (1 2). This test has been found to be relatively simple and reproducible in the hands of various authors, including ourselves (3 4 5 6 7). It gives a good indication of thyroid function, particularly in differentiating between hyper- and euthyroidism. The principle of the method is as follows. To a sample of heparinized blood radioactive triiodothyronine is added and the affinity of the plasma for this hormone is determined by measuring the amount of radioactive hormone which is bound to the erythrocytes. This makes it possible to obtain a crude indication of the saturation of the plasma with bound thyroxine. However the red cell T3 uptake being dependent on a number of extrathyroidal factors, is only an indirect parameter of thyroid function like the other tests in current clinical use. In order to be a valuable clinical tool it should be sufficiently sensitive to indicate the patient's gradual progress towards euthyroidism. The object of the present study is to assess the correlation between the values found and the clinical course in patients with hyperthyroidism and myxedema.

Procedure

A sample of heparinized blood is separated into plasma and erythrocytes. 4 ml erythrocytes are added to 6 ml plasma and shaken gently. Of this 3 ml is pipetted into an open plastic tube and incubated at 37° for half an hour with 0.1 ml of a solution of radioactive triiodothyronine (Abbott) containing 5—20 millimicrogram of the hormone. Then radioactivity is measured and the erythrocytes are washed in saline five times. The erythrocytes are hemolysed with saponin and residual radioactivity is measured.

The red cell T3 uptake is

$$\frac{\text{radioactivity in erythrocytes}}{\text{total radioactivity}} \quad 100 \quad \frac{100}{40} (\quad)$$

The normal range obtained in our laboratory is 6—16

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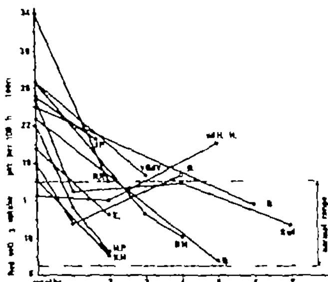


Fig 1 Red cell triiodothyronine uptake in hyperthyroidism before and following treatment with radioactive iodine

● Good correlation with clinical assessment.

No correlation.

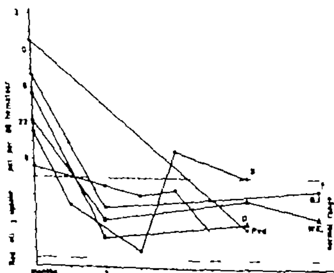


Fig. 2 Red cell triiodothyronine uptake in hyperthyroidism before and following operation ▲ or treatment with methylthiouracil or methimazole ●

● ▲ Good correlation with clinical assessment

No correlation

Fig 2 shows the progress of 2 patients following operation and of 4 while on maintenance therapy with methylthiouracil or methimazole. Table II gives further data concerning these patients. Patient H S. was treated initially with a combination of Lugol's solution and methylthiouracil which resulted in over treatment and temporary worsening of his exophthalmos. Following cessation of Lugol and decrease of the maintenance dose of methylthiouracil he again experienced an exacerbation of his disease with weight loss and palpitations. H continues now to be well controlled on a dose of 100 mg of methylthiouracil.

Table I Data for patients in fig 1

Name	Period since treatment	Pulse rate	Weight (kg)	I^{131} uptake (%) 6 hrs	I^{131} uptake (%) 30 hrs	PBI^{131} (% L) 48 hrs	PBI (μg)	Red cell uptake ( )	Treatment I
R. R.	2 months	160	78	76.5	65.5	3.0	8.5	34	8 mC
		90	79.3					16	
B. M		96	62.5	73	65.5	0.5	16.7	78 6	10 mC
	3 months	72	66.9					12.5	
	4 months	78	—					10.1	
v d G d. V		96	70.5	71	68.3		11.2	27	8 mC
	3 months	72	76.4					16.7	
J P		90	66.5	73	73	0.6		25.0	8 mC
	7 weeks	84	67 4					20.5	
S. vd. S.		120	56.1	92.7	77 9	0.9	10.1	24.9	8 mC
	1 month	—	62					14.8	
	4 month	96	64.5					13.8	
	7 months	90	65					11.5	
K B.		90	77.[illegible]	64.8	72.6	0.2	14.3	24.0	10 mC
	6 months	60	82.2					13.6	
D N		108	60.1	95.9	90.3	2.0	13.3	23	8 mC
	5 months	90	69					7.5	
H P		92	63.8	71	77	0.3		22	9 mC
	1 month	78	66.0					13.2	
	2 months	72	76.5					8.5	
E. V		108	70.6	74.3	69.5	1 6	10.9	19.0	10 mC
	2 months	76	71 3					12.4	
	8 months	66	76.5					—	
K. O		108	58.7	68.5	83.5	0.5	11.5	18	8 mC
	1 month	72						11.5	
	4 months	64	69.3					16.7	
K. H.		120	63.5	71.5	55.5	2.8	8.0	16.2	10 mC
	2 months	72	65.5					8.2	
vd. H. v H		104	62	76.5	64.0	2.6	10	14.6	8 mC
	2 months	120	59.3					14.0	10 mC
	5 months	72	64					20.0	

Results

Diagnosis in the patients studied was based on clinical evaluation combined with radioactive iodine studies and/or determination of PBI Fig 1 shows the progress after treatment with radioactive iodine in 12 patients. Further data are given in table I All patients had a clinical remission with disappearance of sweating tremors tachycardia and a gain in weight. In all cases a fair correlation with the exception of patient v d H. v H We have no plausible explanation for the failure of the method in this patient.

Table III Data for patients in fig 3

Name	Period on treatment	Weight	Pulse rat	I¹³¹ uptake (%)		PBI¹³¹ (%/L) 48 hrs	PBI (μg %)	B.M. %	Red cell uptake (%)	Treatment (pulv gl. thyr.)
				6 hrs	30 hrs					
K.-M.		82	72				3.3	− 3	6.7	75 mg
	7 months	80	72						13.5	150 mg
H.-v.d.T.		77	60				2.5	+ 5	6.7	100 mg
	4 months	78.2	66				2.9		14	150 mg
	8 months	78.3	60						12.2	150 mg
K.-H.		80	56				1.2	+ 12	7.8	50 mg
	3 months	75.3	72				6.1		17	150 mg
L.-v. B.		98.5	60	2.5	6.5	0.18	1.9	− 23	9.2	
	5 months	76.8	60						12.2	150 mg
W.-H.		79	60	4.5	9	0.3	0.8	− 20	9.5	
	7 months	72.9	102				7.4		13	150 mg

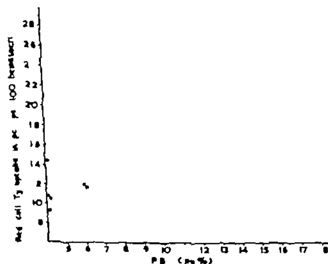


Fig. 4 Red cell triiodothyronine uptake in correlation with PBI

● PBI and red cell T3 uptake correlate with clinical thyroid status.

○ PBI correlates better with clinical thyroid status than red cell T3 uptake

Fig 3 and table III give the findings in patients with untreated or insufficiently treated myxedema. It is here that the test gives results that are disappointing more than half of the cases have initial values well in the normal range.

Unfortunately PBI levels following treatment are only partially available in the patients discussed above. However more recently we found a fair correlation of red cell triiodothyronine uptake with PBI in hyperthyroidism and euthyroidism (fig 4)

Table II Data for patients in fig 2

Name	Period on treatment	Weight	Pulse rate	I[131] uptake (%) 6 hrs	I[131] uptake (%) 30 hrs	PBI[131] (% L) 48 hrs	PBI (g%)	Red cell uptake (%)	Treatment
P vdV		55.2	80	68.2	60.9	1 7	10.2	31	Methimazole
	6 months	60	72					10.5	
G J		61 1	88	67 6	57 4	0.5		27.3	Methylthiouracil
	1 month	—	78					19.7	
	2 months	61.8	78					12.7	
	8 months*	62	72					14 7	
D. H		60.3	112	73.5	29.5	1 7	13.8	25.8	Operation
	2 months	67.5	64					10	
	6 months	73	78					11	
W E.		74	92	65.5	64	0.4	15.4	22.3	Operation
	2 months	85.6	64				1.2	11.3	Pulv gl. thyr
	6 months	86	60				1.9	13.8	
	8 months	77	96				5.8	11 7	
H. S.		71.3	124				16.1	21	Methylthiouracil and lugol
	1 month	79	66					13.0	
	3 months	80.5	82				2.1	7.8	
	4 months	72.5	126					20.8	
	6 months	78	96				8.4	16	
vd. L.		48	128				17.8		Methylthiouracil
	1 month	55.5	80				1_4	17.6	
	2 months	64 7	72				7 0	15.3	
	3 months	71.2	78					14.2	
	4 months	72.6	80					14.4	
	5 months	77 6	72					10.2	

Still having tremor and excessive sweating
Clinically euthyroid.

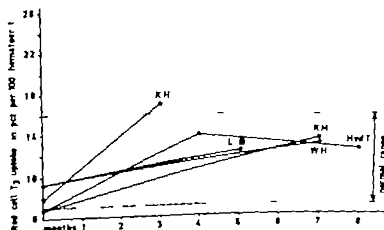


Fig 3 Red cell triiodothyronine uptake before and following adequate treatment of hypothyroidism.

the relative amounts of free and bound radioactive thyroxine added to plasma without changing the pH holds more promise.

Considering the many theoretical pitfalls, it is surprising that the red cell triiodothyronine uptake has proved itself a useful adjunct in the diagnosis of thyroid disease in clinics with adequate isotope facilities.

Summary

The red cell triiodothyronine uptake was followed in a number of patients with thyrotoxicosis (18) and myxedema (5) during their treatment. The test gives an adequate picture of progress in most instances, despite the many theoretical objections that can be raised against it. It should not be used when the diagnosis of myxedema is considered. Since it is, like most of the other tests, only an indirect parameter of thyroid function, it should never be used as the sole criterion in evaluating thyroid status.

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Discussion

There is much evidence that the red cell T3 uptake test depends on the affinity of plasma for circulating thyroid hormone Both thyroxine and triiodothyronine are bound mainly to the thyroxine-binding protein (TBP) and in part to albumin and pre-albumin which in a tris-maleate buffer electrophoresis system separates as a minor fraction Addition of tracer quantities of radioactive triiodothyronine to a heparinized blood sample gives a partition of the radioactive hormone among these three plasma fractions and the erythrocytes. In order to obtain a reproducible partition we have found it necessary to work at a constant hematocrit of 40

The equilibrium in the binding of radioactive triiodothyronine with plasma and erythrocytes changes when disturbances of plasma composition occur as for instance in liver disease, nephrosis, malignancy and following medication with anticoagulants (1) Both metabolic acidosis (uremia, diabetic coma, previous ingestion of ammonium chloride) and respiratory acidosis increase the triiodothyronine uptake by the erythrocytes. In all these instances the red cell T3 uptake falls in the hyperthyroid range

The principal value of the test is that it can be used in cases where I studies are not feasible for instance because of previous iodine ingestion and in pregnancy

In evaluating the reliability of this test for clinical use one should realize that the main determinant of the plasma affinity for thyroid hormones TBP has not yet been isolated Individuals with high levels of TBP and with no TBP at all being euthyroid to all appearances, have been described (8 9) The information obtained with the test concerning thyroid status in these individuals has been shown to be misleading Also it has been demonstrated that the thyroxine binding capacity can be influenced by administration of estrogens (10 11)

The erythrocytes can be replaced by any suspension of small particles, for instance the resin Amberlite CG 19 (12, 13) Thyroxine cannot be used instead of triiodothyronine for measurement of the percentage uptake by the red blood cells, possibly because it conjugates more firmly with the plasma.

Possibly the saturation of plasma with thyroid hormone is the main determinant of the percentage of radioactive triiodothyronine taken up by the red cells. In the present study we did not find any correlation between the red cell uptake and in vitro thyroxine binding capacity of plasma Data concerning thyroxine-binding capacity of plasma are given by Albright et al (14) and Robbins and Rall (15) It can be estimated by adding increasing amounts of thyroxine to plasma and determining the distribution of this exogenous hormone in the TBP and (pre)albumin fractions. It is then found that the amount bound to TBP reaches a plateau, the excess thyroxine added being bound to the secondary carriers pre-albumin and albumin (16) Using tris-maleate buffer at pH 8.6 we found a thyroxine-binding capacity of 64—102 γ % in myxedema, of 30—62 γ % in euthyroidism of 16—60 γ % in thyrotoxicosis. These values are higher than those stated by Robbins and Rall It will be seen that the stated value for in vitro thyroxine-binding capacity of plasma is clearly an in vitro artefact in all thyroid conditions, probably caused by the elevation of pH in the tris-maleate electrophoresis sytem It might be that the method of Christensen (17) measuring

the relative amounts of free and bound radioactive thyroxine added to plasma without changing the pH holds more promise.

Considering the many theoretical pitfalls, it is surprising that the red cell triiodothyronine uptake has proved itself a useful adjunct in the diagnosis of thyroid disease in clinics with adequate isotope facilities.

Summary

The red cell triiodothyronine uptake was followed in a number of patients with thyrotoxicosis (18) and myxedema (5) during their treatment. The test gives an adequate picture of progress in most instances, despite the many theoretical objections that can be raised against it. It should not be used when the diagnosis of myxedema is considered. Since it is, like most of the other tests, only an indirect parameter of thyroid function, it should never be used as the sole criterion in evaluating thyroid status.

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The Insulin-like Activity in Serum Determined by the Rat Epididymal Fat Method

I. Normal Values in Undiluted and Diluted Serum, and the Effect of Ingestion of Glucose

By

Jens Lyngsøe

Serum insulin-like activity (SILA) was first determined in 1941 when Gellhorn showed that injection of human blood into hypophysectomized-adreno-demedullated rats caused a fall in the blood sugar which could be used for the determination of SILA. Since then several studies on SILA have been published (Bornstein 1950) mainly in vitro experiments using the rat diaphragm method (Groen et al. 1952, Vallance-Owen et al. 1954, Randle 1954, Willebrands et al. 1956, Wright 1957, Randle 1957, Seltzer & Smith 1959) and the rat epididymal fat method (Du-rehumett et al. 1959, Sheps et al. 1960). Recently the results obtained with an immunologic method have been reported (Yalow & Berson 1960).

Bornstein and the investigators who used the rat diaphragm method found that SILA generally ranges from about 50 to 100 microunits insulin per ml (µU/ml) in normal fasting subjects. The SILA values are found to rise when glucose is administered. Higher values have been reported by investigators using the rat epididymal fat method, but only few results obtained with this technique have been published. In addition the temperature at which blood is stored before incubation has been found to affect SILA values determined by the rat epididymal fat method (Lyngsøe 1961 a).

The SILA values have been shown to be higher in diluted than in undiluted serum (Randle 1957, Bergelman et al. 1958, Willebrands et al. 1958, Wright 1960) but only few data elucidating this "dilution effect" are available.

In the present paper results are reported from a series of investigations of SILA values in normal subjects determined by a modified rat epididymal fat method (Lyngsøe 1961 a). Fasting subjects and subjects to whom glucose was administered were examined, and SILA was determined in undiluted as well as in diluted serum.

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mean value 70.6 μU/ml

number of normal subjects

10

5

100

undiluted serum

mean value 173.7 μU/ml

number of normal subjects

10

5

100 200 300 400 μU/ml

450 555 605

diluted serum

Fig. 1. Distribution of normal values of SILA in undiluted and diluted fasting serum.

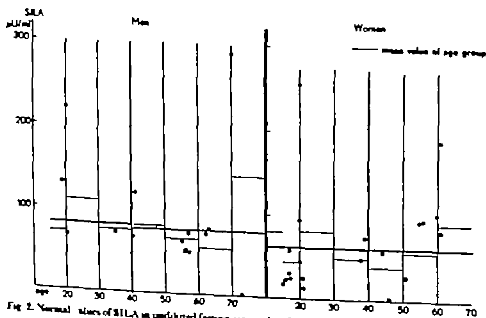


Fig. 2. Normal values of SILA in undiluted fasting serum distributed according to age and sex.

Technique

Serum is made from blood drawn from a slightly stased vein of the upper extremity. Immediately after the drawing of the blood it is stored at 4° C for *30 to 60 minutes*, and subsequently spun at room temperature for 10 minutes at 3 000 r.p.m. The coagulum is detached by a wooden stick and re-spun for 5 minutes at 3 000 r.p.m. Serum is pipetted off and stored at 4° C until incubation, i. e. 4—6 hours after the blood was drawn.

The modification of the original epididymal fat method has been described in an earlier paper (Lyngsoe 1961 a). The index of precision and the SILA values are calculated as reported in this paper. SILA values below 10 μU/ml are recorded as 0 μU/ml. The glucose concentration in serum was determined by the glucose oxidase method (Huggett & Nixon 1957).

Material

The series includes patients from the Department of Neurology of the Århus Kommunehospital; none of these patients was suffering from diseases accompanied by glycosuria or high blood sugar. The majority of the patients were admitted suffering from hemicrania, spondylosis or neurosis. None had diseases attended by fever or malignant tumours. Fasting serum glucose was below 105 mg % in the sera used for SILA determinations, and glycosuria was not observed in the morning urine examined with Clinistix.

Experimental conditions

The day preceding the examination the subjects were allowed their usual diets; they did not leave bed during the night. Blood was obtained by venipuncture between 7 and 8 a. m., i. e. after fasting periods of about 8—12 hours. In patients who were examined both in the fasting state and after glucose administration blood samples were drawn as described above. Glucose was then administered orally (1 g/kg body weight). The patients stayed in bed throughout the examination. 90 minutes after administration of glucose the venipuncture was repeated.

Results

SILA values in serum from fasting normal subjects

Findings from examinations of SILA in *undiluted* serum are shown in fig. 1 which shows the *number of subjects* whose SILA values come within the individual 20 μU/ml intervals. The arithmetical mean of the 73 fasting subjects was found to be 70.6 μU/ml, the extreme values being 0 and 290 μU/ml. Values between 0 and 140 μU/ml were seen in 90 % of the cases.

In fig. 2 the SILA values are classified according to age and sex. The arithmetical mean in the age groups 10—19, 20—29, 30—39, 40—49 etc. is calculated in males as well as in females and is represented by horizontal lines. The arithmetical mean values are calculated also for the groups males and females over and under the age of 40 years, and these values are represented in the figure by horizontal lines. It will be noted from the distribution of the various arithemetical mean values, that neither age nor sex variations are manifest in the present material.

Findings from examinations of SILA in *diluted serum* (20 % serum) are seen in fig. 1. SILA values in diluted serum range between 0 and 605 μU/ml. The arithmetical mean of the 73 fasting subjects is found to be higher in diluted than in undiluted serum, viz. 173.7 μU/ml. In 90 % of the cases the values were found to range between 0 and 340 μU/ml.

In fig. 3 the values are classified according to age and sex, as for the values with undiluted serum. The arithmetical mean of values in female subjects tends to be lower than that in male subjects, particularly in the younger age groups; the arithmetical mean values of female and male subjects in the age group *under*

mean value 70.6 μU/ml

number of normal subjects

10

5

100

undiluted serum

mean value 173.7 μU/ml

number of normal subjects

10

5

450 555 605

100 200 300 400 μU/ml

diluted serum

Fig. 1. Distribution of normal values of SILA in undiluted and diluted fasting serum.

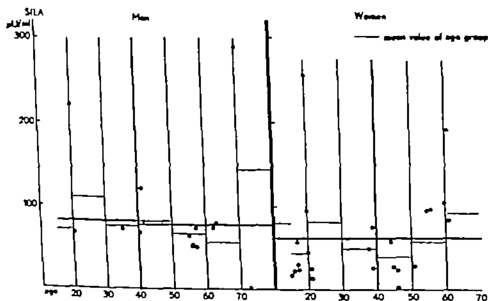


Fig. 2. Normal values of SILA in undiluted fasting serum distributed according to age and sex.

Technique

Serum is made from blood drawn from a slightly stased vein of the upper extremity. Immediately after the drawing of the blood it is stored at 4° C for 30 to 60 minutes, and subsequently spun at room temperature for 10 minutes at 3,000 r.p.m. The coagulum is detached by a wooden stick and re-spun for 5 minutes at 3,000 r.p.m. Serum is pipetted off and stored at 4° C until incubation, i. e. 4—6 hours after the blood was drawn.

The modification of the original epididymal fat method has been described in an earlier paper (Lyngsøe 1961 a). The index of precision and the SILA values are calculated as reported in this paper. SILA values below 10 μU/ml are recorded as *0 μU/ml*. The glucose concentration in serum was determined by the glucose oxidase method (Huggett & Nixon 1957).

Material

The series includes patients from the Department of Neurology of the Århus Kommunehospital; none of these patients was suffering from diseases accompanied by glycosuria or high blood sugar. The majority of the patients were admitted suffering from hemicrania, spondylosis or neurosis. None had diseases attended by fever *or malignant tumours. Fasting* serum glucose was below 105 mg % in the sera used for SILA determinations, and glycosuria was not observed in the morning urine examined with Clinistix.

Experimental conditions

The day preceding the examination the subjects were allowed their usual diets; they did not leave bed during the night. Blood was obtained by venipuncture between 7 and 8 a. m., i. e. after fasting periods of about 8—12 hours. In patients who were *examined both* in the fasting state and after glucose administration blood samples were drawn as described above. Glucose was then administered orally (1 g/kg body weight). The patients stayed in bed *throughout the* examination. 90 minutes after administration of glucose the venipuncture was repeated.

Results

SILA values in serum from fasting normal subjects

Findings from examinations of SILA in *undiluted* serum are shown in fig. 1, which shows the number of subjects whose SILA values come within the individual 20 μU/ml intervals. The arithmetical mean of the 73 fasting subjects was found to be 70.6 μU/ml, the extreme values being 0 and 290 μU/ml. Values between 0 and 140 μU/ml were seen in 90 % of the cases.

In fig. 2 the SILA values are classified according to age and sex. The arithmetical mean in the age groups 10—19, 20—29, 30—39, 40—49 etc. is calculated in males as well as in females and is represented by horizontal lines. The arithmetical mean values are calculated also for the groups males and females over and under the age of 40 years, and these values are represented in the figure by horizontal lines. It will be noted *from the distribution of the* various arithmetical mean values, that neither age nor sex variations are manifest *in the present material.*

Findings from examinations of SILA in *diluted serum* (20 % serum) are seen in fig. 1. SILA values in diluted serum range between 0 and 605 μU/ml. The arithmetical mean of the 73 fasting subjects is found to be higher in diluted than in undiluted serum, viz. 173.7 μU/ml. In 90 % of the cases the values were found to range between 0 and 340 μU/ml.

In fig. 3 the values are *classified* according to age and sex, as for the values with undiluted serum. The arithmetical mean of values in *female subjects* tends to be lower than that in male subjects, particularly in the younger age groups; the arithmetical mean values *of female* and male subjects in the age group under

Table II. SILA before and after glucose administration to normal subjects

Assay No.	Normal subject No.	Serum glucose before and after glucose administration mg %	SILA in undil. serum		SILA in dil. serum		Index of precision	No. of rats
			Fasting μU/ml	90 min. after glucose μU/ml	Fasting μU/ml	90 min. after glucose μU/ml		
165	63	95—102	73	154	85	255	0.21	4
167	59	94—116	160	190	185	525	0.32	3
175	69	104—137	122	225	145	155	0.24	3
179	71	85—95	93	105	180	270	0.21	4
181	73	82—88	55	78	79	400	0.25	4
182	74	84—101	64	68	95	80	0.08	3
183	75	91—186	23	520	130	750	0.33	3
189	81	89—99	27	82	100	73	0.34	3
190	82	84—114	0	0	65	60	0.33	4
191	83	87—137	95	330	0	340	0.22	4
192	84	81—113	45	150	0	113	0.31	4
277	175	95—125	110	200	350	450	0.25	4
278	176	97—134	88	240	270	410	0.23	4
281	179	96—124	27	94	100	85	0.26	3
282	180	103—130	52	48	150	215	0.18	4
286	184	96—140	44	66	120	175	0.20	4
Arithmetical mean "Group I"			67.5	159.4	128.4	272.5		
172	66	88—88	65	25	55	95	0.31	3
174	68	100—96	13	17				3
181	76	98—99	50	85	118	475	0.28	4
168	69	91—79	90	240	325	483	0.43	3
177	54	91—82	68	130	375	720	0.38	3
180	72	89—69	40	54	280	270	0.33	3
187	79	84—68	27	21	605	650	0.24	3
Arithmetical mean "Group II"			50.4	81.4	279.7	449.2		
Arithmetical mean of all subjects examined "Group I" + "Group II"			62.3	133.7	169.6	320.6		

0.297 ± 0.082. In 30 experiments carried out in quadruplicate the average lambda value was 0.262 ± 0.077.

Overweight subjects

Overweight was defined as ≥ 115 per cent of the "ideal weight" based on the "Hafnia table" in which the average weights of normal Danish individuals are recorded. The average male and female weights in the age group 30—34 years in this table have served as "ideal weight". However, in the above table the weights refer to fully dressed individuals whereas the subjects included in the present material were weighed without clothes; consequently the registered weights have been reduced by 1.5 and 1 kg for males and females respectively.

Table III records SILA values in fasting serum obtained from 7 subjects who according to the above definitions were

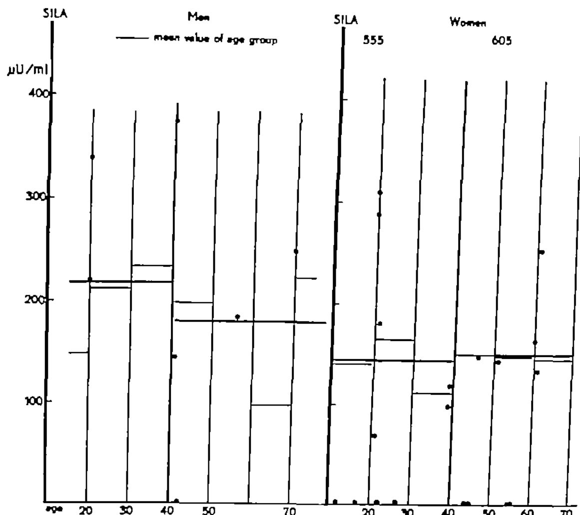


Fig 3 Normal values of SILA in diluted fasting serum distributed ccording t age nd sex.

Table I SILA in fasting serum from overweight normal subjects

Subject No.	Sex	Weight •	SILA in undiluted serum μU ml	SILA in diluted serum μU ml	λ	No. of rats
60	M	115	90	325	0.43	3
66	M	126	65	55	0.31	3
83	F	132	95	0	0.22	4
95	F	134	97	450	0.30	4
137	M	115	75	385	0.25	4
139	M	123	75	85	0.30	4
140	M	119	80	130	0.32	4
Arithmetical mean			82.4	204.2		

40 years are 144.8 μU/ml and 220.7 μU/ml respectively but the difference is not statistically significant ($0.05 < p < 0.1$). Thus neither age nor sex variation is demonstrated in the present material of SILA values in diluted serum from fasting subjects. No relation could be demonstrated between the fasting SILA values in undiluted or diluted serum and the fasting serum-glucose values.

The *index of precision* is calculated for the 56 experiments included in these determinations of SILA in serum from fasting subjects. In 26 experiments in which adipose tissue from 3 rats was used the lambda value was found to be

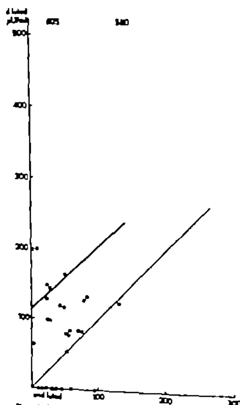


Fig 4 Relation between SILA alues in undiluted and diluted fasting serum. Regression coefficien (by 0 915 = 2 103 p < 0.05.

I the present material of normal subjects the rise in SILA values pon administration of glucose seems to be independent of the level of fasting SILA alues.

A comparison of groups I and II (cf table III) show that the rise i SILA lues in dil ted serum after administration of glucose is of the same order of magnitude in the two groups.

It is interesting t not that the a erage fasting alues of SILA are higher group II than in group I ($p < 0.05$)

Dilution effect

The relation between SILA alues in dil ted and undiluted sera is illustrated

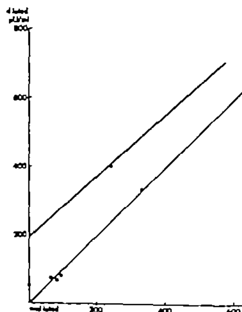


Fig 5 Relation between SILA alues in undiluted and diluted serum drawn 90 minutes after glucose administration. Regression coefficient ($b_{y.x}$) = 0.915 t = 2.877 $p < 0.01$

in figs. 4 and 5 The SILA values are higher in diluted than in undiluted serum in 58 out of 73 fasting sera. The same is seen in 18 out of 22 sera obtained after administration of glucose It also ppears that there is a correlation between the SILA alues in undiluted and diluted sera. In fasting serum this correlation can be shown only in the area 0—140 μU ml ($p < 0.05$) In sera obtained after

Table II Dilution effect SILA in diluted serum — SILA in undiluted serum

	Mean U ml	Standard deviation	No of sera	P
Fasting sera	103 1	132	73	< 0 001
Sera dra 90 min. after glucose administration	179.6	189	22	< 0 001

Table III

a. Statistical calculation of SILA before and after glucose administration to normal subjects

Undiluted serum	No. of subjects examined	SILA fasting mean standard error μU/ml	SILA after glucose mean standard error μU/ml	Rise in SILA mean standard error μU/ml	t	p
Group I	16	67.5 10.5	159.4 22.1	91.9 31.5	2.936	< 0.01
Group II	7	50.4 10.0	81 4 44.2	31 0 23.2	1 336	> 0.1
All normal	23	62.3 8.0	135.7 25.0	73.4 23.4	3.137	< 0.01
Diluted serum						
Group I	16	128.4 22.4	272.5 48.7	144 1 44.5	3.238	< 0.01
Group II	6	279 7 81 7	449.2 109	169.5 60.1	2.820	< 0 05
All normal	22	169.6 30.2	320.6 46.2	151 0 35.6	4.241	< 0.001

b. Differences between group I and group II

	Undiluted serum		Diluted serum	
	t	p	t	p
Fasting values	0.979	> 0.1	2.509	< 0 05
Values after glucose	1 473	> 0.1	1 792	< 0.1
Rise after glucose	1 150	> 0.1	0 310	> 0.1

found overweight The values determined were found to correspond with those obtained in the rest of the material.

Effect on SILA of administration of glucose

Twenty three subjects were examined in the fasting condition as well as after oral administration of glucose. Findings are illustrated in table II *In 19 out of 23 subjects* SILA values determined in undiluted serum were found *to rise 90 minutes* after administration of glucose In the entire material the average rise was found to be 73 4 μU/ml This rise is statistically significant ($p < 0\ 01$) No correlation between fasting SILA values determined in undiluted serum and increases in *SILA* after glucose administration could be demonstrated in this material

In some subjects the serum glucose had fallen to fasting levels within 90 minutes, in others the fall was less rapid Consequently the SILA values were divided into two groups group I including sera from subjects in whom the serum glucose exceeded the fasting value by 5 mg 90 minutes after administration of glucose and group II including sera from subjects in whom the serum glucose value fell below this level within the same time. The average SILA value in *undiluted serum* after administration of glucose in group I (16 cases) was 91 9 μU/ml *higher than the fasting value* (table III a. $p < 0\ 01$) In group II (7 cases) *the average value of SILA in undiluted* serum was also found to have increased *after administration of glucose* but the rise was less pronounced (viz 31 0 μU/ml) *and not statistically significant* ($p < 0\ 1$)

In *diluted serum* the average rise in SILA values *after administration of glucose* was 151 0 μU/ml in all the cases examined

1960) shows that normal values found by the latter method are much lower than values found by any other method. The reason for this difference remains to be explained, but recent studies have suggested that the total insulin content in plasma is not determined by the immunological method because protein-bound insulin does not react in this system (Anthoniades et al. 1960).

The normal SILA values in diluted fasting sera obtained in the present study are of the same order of magnitude as values found by Wright (1960) who used the rat diaphragm method. Higher values were found by Randle (1954) and Willebrands et al. (1956) but this is perhaps explained by the inadequacy of the modification in the rat diaphragm method used by these investigators (Piazza et al. 1960). Diluted serum has been examined only in few cases by the rat epididymal fat method. Steink & Renold (1960) found values in diluted serum to be higher than values found in the present material, but this feature seems to be caused by a difference of procedure (Lyngsøe 1961). Duchunent et al. (1959) found higher values than those shown in the present paper in serum dil ted by 50 %, but the technique by which serum was mad is not described.

Neither age nor sex ... rations in SILA values in d'l ted fasting serum were demonstrable in the present work, but values tend t be lower in females than in males, most pronouncedly in the age groups under 40 years. SILA values in overweight subjects were of the same order of magnitude as in normal subjects. Ninety minutes fter administration of glucose the SILA ... lues in diluted serum were seen t rise the the rise was almost to twice the fasting values.

It has been demonstrated by previous investigators who used the rat diaphragm method that SILA values in diluted serum exceed values in undiluted serum (Randle 1957, Beigelman et al. 1958, Willebrands et al. 1958, Wright 1960). The same result was obtained in 76 of 95 sera in the present work using the rat epididymal fat method. Steinke & Renold (1960) failed to demonstrate a significant rise on dilution of serum from 7 normal subjects.

It has been suggested that the rise in SILA values caused by dilution is due to elimination of a factor which is antagonistic to the insulin effect. Vallance-Owen has demonstrated a pituitary dependent insulin antagonist in albumin fractions from normal subjects and from diabetics: the author suggested that dilution of serum might eliminate the effect of this antagonist (Vallance-Owen & Wright 1960). Studies of serum from hypophysectomized, insulin-treated diabetics show however that the dilution effect may be demonstrated in such serum by means of the rat epididymal fat method (Lyngsøe 1961 b).

Groen et al. (1958) found that epinephrine inhibits the insulin effect on glucose uptake in the rat diaphragm and also that this effect can be eliminated by dilution. Hence the authors concluded that the increase n SILA observed after dil tion might be ascribed to an elimination of the effect of epinephrine. However studies are available from which it appears that such oncentrations of epinephrine as are actually present in serum do not inhibit the insulin effect on the glucose uptake or on the conversion of glucose-1-C^{14} into $C^{14}O_2$ in rat epididymal tissue (Hagen et al. 1960, Cahill et al. 1960, Renold et al. 1960).

Recent investigations suggest that insulin is present in serum partly in a complex form, probably bound to pr tein

administration of glucose an interdependence appears in the entire area 0—520 μU/ml ($p < 0.01$)

The "dilution effect" is calculated for all the sera included as the difference between SILA values in diluted and undiluted serum Table IV demonstrates that the "dilution effect" differs significantly from zero in fasting serum as well as in serum obtained after administration of glucose. The "dilution effect" seems to be higher in serum drawn after glucose administration, but there is no statistically significant difference between the two arithmetical mean values ($0.05 < p < 0.1$)

Discussion

The *normal values* for SILA obtained in undiluted serum from fasting subjects are of the same order of magnitude as the values found by Bornstein (1950) who used the ADHA rat method and by Vallance-Owen et al (1954) Wright (1957) and Seltzer & Smith (1959) who used the rat diaphragm method

Neither age nor sex variations have been demonstrated in the present material and the SILA values found in overweight subjects are not different from those found in subjects of normal weight

Values in undiluted serum from normal subjects reported by other investigators (Sheps et al 1960) using the rat epididymal fat method are higher than those found in the present investigation The small differences between the procedures used by Sheps et al and those used in the present study are discussed in an earlier paper (Lyngsoe 1961 a) Most important is probably the fact that serum is prepared by different techniques. Sheps et al left blood to coagulate for 2 hours at room temperature after which serum was stored in a deep-freezer In the present work blood is kept at 4 C spun at room temperature, and kept at 4 C until incubation (4—6 hours after being drawn) This difference of procedures is important By imitating closely the procedures of Sheps et al. we have obtained higher values than those reported in the present paper (Lyngsoe 1961 a) The reason why SILA values are higher when serum is made at room temperature and then frozen remains to be explained. Either an insulin antagonist may be decomposed or insulin may be liberated from a plasma protein (Gundersen & Anthoniades 1960)

Ninety minutes after oral administration of glucose a rise in SILA values was observed In undiluted serum the values rose two-fold. In other studies in which the rat diaphragm method has been used (Vallance-Owen et al. 1954 Seltzer & Smith 1959) the value attained averaged about 6 to 10 times the original value. In these works, however serum was examined 1 hour after the oral administration of glucose i. e. at a time when the glucose values will usually be higher than after 90 minutes By continuous infusion of glucose solutions it has been shown that the insulin production is proportional to the blood glucose level (Metz 1960) This may explain why values after administration of glucose were found to be lower in the present work than in the other two studies It is also in accordance with the fact that our SILA values in undiluted serum rose most noticeably in subjects in whom the blood sugar had not yet fallen to the original values at the time when the second blood samples were drawn

A comparison of previously published SILA values in undiluted sera and values obtained by the newly published immunological method (Yalow & Berson

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YALOW R. S. & BERSON, S. A. J Clin Invest. 39 1157 1960

in this form it is not capable of accelerating the glucose uptake in rat diaphragms (Gundersen & Anthoniades 1960) But complex bound insulin does accelerate the oxidation of glucose-1 C^{14} into $C^{14}O_2$ in adipose tissue. These results, however, remain to be elucidated by quantitative investigations It is possible that dilution may liberate insulin which affects the adipose tissue to a higher degree than does the insulin-protein complex.

In all of the subjects the insulin like activity in diluted serum was seen to rise after administration of glucose.

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Summary

In a series of 73 normal subjects an average insulin-like activity of 70 6 μU/ml (< 10—290 μU/ml) has been found in undiluted fasting serum by means of the epididymal fat method These values are lower than those reported by Renold et al the difference may be ascribable to variations in the experimental conditions.

In fasting serum diluted with buffer in the proportion 1 5 a higher insulin like activity was found viz. an average of 173 7 μU/ml (< 10—605 μU/ml)

Neither age nor sex variations were demonstrated in the present material 7 overweight subjects presented the same values as the rest of the normal material

Ninety minutes after administration of glucose to 23 subjects the insulin like activity rose significantly in undiluted serum from subjects in whom the serum glucose values at that time still exceeded the fasting values by more than 5 mg % In subjects in whom the 90-minute blood sugar was lower the average value also exceeded the values obtained before administration of glucose but the difference was not statistically significant.

Acknowledgement

I wish to express my gratitude to H C. Hagedorn, M D Nordisk Insulinlaboratorium, for an ample supply of Glucagon free insulin, and to Miss Tove Dreyer Petersen for her skilful technical assistance.

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Chapter 8 and 9 describe pulmonary abscess and bronchiectasis — both very indicative forms of pulmonary pathology for the bronchologist.

In chapter 10 tuberculosis and mycosis are mentioned very shortly and superficially but the description of the tumours (in chapter 11) is good and informative reading, and the twelwth chapter concerning foreign bodies is perhaps the best chapter with lots of good histories, X-rays and almost a museum of foreign bodies.

This might have been the end of the book but the author gives a last chapter with differential diagnosis.

At last a selected list of references is given. The author demonstrates by this his European origin and thus gives the reader a broader view of European literature than usually seen.

Paul Jorgen Dragstad

Copenhagen

Clinical applications of bronchology By Dezzo Kassay with foreword by Chevalier L. Jackson 225 pp 116 fig Price £5 16s. McGraw Hill Publishing House, London 1960

As the title is not too familiar to Scandinavians, especially not the term bronchology one looks very earnestly through the foreword and enters the first chapter but nowhere a definition is given. We can of course figure it out, but would have liked to see it with the author s own phrases After having read the book — nevertheless — nobody is left in doubt what the author feels The subtitle might have been the world seen through a bronchoscope — all chapters also the ones which give reference to different pathologies of the lungs are only seen through the mirror of the bronchoscope. This is a new and very refreshing way of keeping up ones knowledge but decreases the value of the book as a textbook. I have a very definite feeling that no practitioners are going to remove foreign bodies from the bronchies after having read the book but this is what the author states in his foreword the book is written for prac titioners — with the addition and other physicians interested in bronchology The author is familiar with his theme and all chapters give in many instances his personal views This already starts in the first chapter The anatomy of the lungs, which is a very valuable chapter for all pulmologists The chapter ends as all other chapters with a summary — an appetizer well formed for the idle

The second chapter treats the physiology very superficially and you will have to look up pulmonary function tests in other books (Comroe etc.) because only the basic facts are given — although the summary is more than two pages.

The third chapter the roentgenology is very well disposed and the discussion of Holzknechts sign (the mediastinal shift) is excellent As in all chapters the X ray pictures are highly illustrative.

In the fourth chapter the types of instruments are described, unnecessary much space is used for the technique of anesthesia and the discussion of local or general anesthesia In the same chapter the author discusses the indications shortly given as nearly all unexplained chest diseases and absolute indications are stenosis purulent sputum hemoptysis and foreign bodies. The author finds practically no contraindications not even aneurysm

And then starts the survey of pathologic conditions, in chapter 5 valvular respiratory mechanisms (cysts, swelling after tracheotomy trauma of the bronchial tree) in chapter 6 infectious and allergic bronchitis, which could have been omit ted. The use of bronchial lavage through the bronchoscope once or twice a week is recommended as the treatment for acute and chronic bronchitis, but it sounds as a drastic procedure

In chapter seven pneumonia is surveyed, especially the middle lobe syndrome (named by Graham in 1948) This special form of pneumonia might be an indication for bronchoscopy to avoid the chronic forms.

Chapter 8 and 9 describe pulmonary abscess and bronchiectasis — both very indicative forms of pulmonary pathology for the bronchologist.

In chapter 10 tuberculosis and mycosis are mentioned very shortly and superficially but the description of the tumours (in chapter 11) is good and informative reading and the twelwth chapter concerning foreign bodies is perhaps the best chapter with lots of good histories, X-rays and almost a museum of foreign bodies.

This might have been the end of the book but the author gives a last chapter with differential diagnosis.

At last a selected list of references is given. The author demonstrates by this his European origin and thus gives the reader a broader view of European literature than usually seen.

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Copenhagen

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From the Medical Clinic (Head: P. Brummer M. D.) the University Turku, Finland

Effect of Intramuscular Cobalt on the Anemia of Patients with Pyelonephritis

By

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The pathogenesis of anemia which occurs in connection with kidney diseases is still unknown. It was formerly attributed to the effect on the bone marrow of toxic substances which accumulate in the organism in azotemia. It has been shown, however, that every third case of anemia in pyelonephritic patients occurs already months, even years, before any retention is seen (Kasanen and Salmi 1959). Nor do retention substances demonstrable in renal insufficiency cause inhibition of bone marrow experimentally (Gardner 1953).

It has been shown experimentally that the erythropoietic factor disappears from plasma after nephrectomy (Goldwasser et al. 1958, Naets 1960). No reticulocyte response to blood transfusions is elicited in nephrectomized animals (Jacobson et al. 1959). It is consequently considered that the kidney is the major if not the sole site of production of the hormone erythropoietin (Jacobson et al. 1960). The release of the erythropoietic factor can be inhibited by a course of X-rays (Ormes 1959). Polycythemia is often established in connection with renal diseases (Forssell 1958). Experimental data on polycythemic renal patients indicate that the kidney is involved with the elaboration and/or metabolism of an erythropoietic factor (Ways et al. 1961).

Erythropoietic factor is associated in some way with the presence of cobalt. The kidney produces an inactive precursor in response to activation by cobaltous ion (Jacobson et al. 1960).

On the other hand, cobalt has been used earlier for the treatment of renal anemia. Seventeen patients with anemia associated with renal disease were treated for 4 weeks or longer with oral cobaltous chloride. Increased erythropoiesis was observed during the period of treatment (Gardner 1953). It has also been administered intramuscularly (Weissbecker 1951). Not all investigators believe that cobalt affects anemia in renal diseases (Sarre 1959).

Recent studies of the erythropoietic factor have thrown new light on anemia in renal diseases, associating cobalt with the process, and earlier clinical observa-

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Table I The effect of cobalt therapy on hemoglobin, red cells and reticulocytes

	Hemoglobin g %	Erythrocytes million/mm	Reticulocytes %
Before therapy	8.4 ± 0.3	3.00 ± 0.12	1 4 ± 0.3
A week after therapy	9.3 ± 0.3	3.02 ± 0.11	3.6 ± 0.5
A month after therapy	9 7 ± 0.4	3.45 ± 0.15	2.4 ± 0.4

tions have suggested the therapeutic possibilities of cobalt. Hence the present authors treated 40 patients with cobalt For the sake of consistency a single renal disease, the pyelonephritis group was selected since it has been shown earlier that the oral administration of cobalt improves the mild anemias associated with chronic suppurative infection (Robinson et al. 1949 Nyrke 1953)

Material

The patients were selected from the Medical Clinic of the University of Turku during the years 1960—1961 All of them were diagnosed as suffering from chronic pyelonephritis. There were 36 women and 4 men. Patients who were known to have had a primary hematopoietic disease or in whom hemorrhage was established in hospital were not included. The mean age of patients was 46 years.

The patients were given an organic complex compound of cobalt (Cobalt Nordmark) intramuscularly every day or every other day for a total of six times. The daily dose was 3 mg of cobalt the total dosage 18 mg of cobalt Blood samples were taken before therapy and 1 week and 1 month after its initiation. Neither iron nor blood transfusions were given to the patients during therapy

Results

The serum iron was determined to rule out iron deficiency anemia. Its mean was 80 mg %. Ten of the patients had received an earlier course of intramuscular iron without success.

Hemoglobin

The mean of the hemoglobin value rose from 8 4 g % to 9 3 g % within a week of the beginning of treatment i. e. by 0 9 g % An increase occurred in three-fourths of the cases. The greatest increase was + 4 4 g One patient who died 1 month after the institution of therapy displayed a 3 1 g % fall.

The mean hemoglobin increase 1 month after the institution of therapy was 1 3 g % (t = 2 6) (table I) Eight cases showed a reduced hemoglobin value during treatment the decrease, however was small within the range of error of the method, averaging 0.3 g % In 14 of the 32 cases in which the anemia was brought under control the increase was over 1 5 g % In 9 of these cases the increase exceeded 2 g % and in 5 was even over 3 g %. The greatest increase in hemoglobin was 6 1 g %

Whereas the initial value was under 10 5 g % for all the patients, it rose above this level in 11 patients after the termination of therapy Hemoglobin was over 12 g % in 3 patients, in 1 of them more than 13 g %. The therapeutic result was not affected by the result of the therapy for the infection.

Erythrocytes

The mean erythrocyte count at the initiation of therapy was 3 000 ± 0.12 million Hardly any increase was demonstrable after a week but after a month the mean rose to 0.45 million (t = 2 6) The increase in erythrocytes was recorded in 29 patients The greatest increase was 1 9 million A fall was demonstrated in

Table II The effect of the severity of the disease on the therapeutic result

	Serum creatinine in mg %				
	< 1.4	1.5—5.0	5.1—10.0	> 10	Total
Responds to therapy Increase in Hg > 1.0 g %	5	11	5	4	25
Does not respond to therapy Increase in Hg < 1.0 g %	4	9	1	1	15
Total	9	20	6	5	40

5 cases only less than 0.3 in all of them, i. e. within the range of error of the method. The therapeutic result was not affected by the state of the infection. The total erythrocyte count rose by over 1 million in a total of 5 cases.

Reticulocytes

Reticulocytosis was small in the majority of the patients, but there were some cases in which anemia involved hemolytic features and reticulocytosis was pronounced. The reticulocyte mean before therapy was 1.4 %. The figure was over 3 % in 4 cases. A significant increase in the reticulocyte count, 2.2 % (t = 3.7) occurred after week. There were 9 patients with reticulocytosis of over 5 % after a week. The greatest increase in the number of reticulocytes was 1.2—8.2 %. No significant reticulocytosis could be seen after a month, but the mean, 2.4 was higher than the initial value.

Effect of the retention of non-protein nitrogen on the therapeutic result

If the diseases are divided into different degrees of severity by serum creatinine it will be seen that the result shows no great dependence on the degree of renal disease (table II). For instance, of the 5 patients whose creatinine exceeded 10.0 mg % at the institution of therapy 4 responded very well to treatment. The hemoglobin of a woman of 63 rose within a month from 4.7 to 8.2 g and her serum creatinine was 11 mg % at the institution of therapy. The reticulocytosis achieved was, surprisingly not dependent on the degree of renal damage. In the patient who showed the greatest increase in reticulocytes 1.2—8.2 % the serum creatinine was 6.6 m

Side effects

Cobalt can cause nausea dyspepsia and also skin symptoms (Kugelmass 1959). The long-term use of cobalt is also known to cause substernal pain and therefore cannot be recommended for patients with coronary disease (Gardner 1953). Tinnitus and vague impairment of hearing have also been reported in 1 patient (Gardner 1953).

The side effects were relatively slight in the present series. Some complained of mild pain at the injection site but no abscess formation was seen. One of the patients developed an intense rash accompanied by pruritus. It has continued for 5 months and no plausible reason has been found to account for it. No intestinal or cardiac symptoms were established.

Discussion

The results seem to indicate that cobalt has an effect on anemia in patients with pyelonephritis. Only a little over half the patients responded to treatment, but the therapeutic result was surprisingly good in some individual cases For instance, one of the patients who had previously received blood every tenth day for 6 months because of rapidly developing anemia was able to dispense with blood transfusions entirely after cobalt therapy and the patient's hemoglobin has remained at 11 0 g % A probable reason why not all patients respond to therapy is that many factors contribute to the genesis of renal anemia. The infection the use of phenacetin and hemolysis each play a part in the genesis of anemia.

It has been shown in animal experiments (Naets 1960) that bone marrow responds to erythropoietic factor even in nephrectomy or uremia. The fault is obviously not in the hematogenic organ but in the missing stimulus. Treatment of the infection does not itself correct the anemia of pyelonephritic patients (Kasanen et al. 1961)

The mechanism of effect of cobalt is still unclarified. It must be regarded as a nonspecific erythropoietic stimulant rather than as a specific nutritional component required for red cell or hemoglobin production. These observations are consistent with the suggestion that cobalt interferes with the transport of oxygen in the erythroid cells of the marrow by binding sulfydryl or perhaps other groups active in cellular respiration thus leading to a cellular anoxia and in turn to a compensatory polycythemia (Orten et al. 1948) The large-scale dosage of cobalt in test animals caused true polycythemia accompanied by reticulocytosis in the blood, hyperplasia in the bone marrow and increased eryhropoietic activity in the spleen and liver Cobalt deficiency had no effect on hemopoiesis (Kugelmass 1959)

Cyanocobalamine is the only cobalt compound which has been isolated in the organism, but vitamin B_{12} does not affect renal anemia. The daily requirement of cobalt for hemopoiesis is less than a microgram, but the therapeutic doses are measured in milligrams. Thus there is no question of correcting nutritional anemia. The effect is in fact based on the inhibiting influence of cobalt on enzyme activity or on its known ability to activate the erythropoietic factor

Blood transfusion has been the only therapy so far in the treatment of anemias of renal origin In order to keep the hemoglobin at the desired level, however transfusions should be given every other week. Blood transfusion must be considered dangerous for renal patients since it can cause lethal pulmonary edema (Kasanen 1958) This is obviously due to capillary osmotic changes in the lungs, and blood is the only fluid which impairs respiratory function in renal patients (Kasanen et al. 1961) The present results with the administration of cobalt are sufficiently encouraging to warrant the trial of cobalt therapy either per os or in injections, against uremias provided a certain care is observed Although the blood is rarely corrected to normal in the way that can be achieved with iron therapy in iron deficiency anemias, a 2—3 g % increase in hemoglobin means nevertheless a distinct improvement in the patient's activity and spirits. Cobalt does not change the basic prognosis of pyelonephritis its value is only symptomatic.

Summary

Forty chronic pyelonephritic patients whose mean hemoglobin prior to therapy was 8.4 g % were treated with a daily intramuscular dose of 3 mg of cobalt for six doses. The blood picture was observed 1 week and 1 month after therapy. The mean hemoglobin rose during therapy by 1.3 g % in the total series, and by over 2 g % in 9 patients. The red cell count rose by 0.45 million. The most significant increase was that in reticulocytes, 1.4—3.6 % after a week. The therapeutic result was not dependent of the severity of the renal disease. It was possible to achieve a good therapeutic result even with serum creatinine values of over 10 mg %.

Side effects were few. One patient developed a severe rash and pruritus.

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Lupoid Hepatitis

By

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The LE-cell phenomenon was first described in liver disease by Joske and King in 2 young women with active chronic hepatitis (8). In several other cases of chronic hepatitis they found nucleophagocytosis and erythrophagocytosis. Mackay, Taft and Cowling reported positive LE-cell phenomena in 14 patients with chronic hepatitis (9, 10). They drew attention to the similarity in sex and age distribution in their patients to systemic lupus erythematosus (S. L. E.). Other features of S. L. E. were arthralgias, epistaxis, rashes, involvement of serous membranes, acne and menstrual irregularities, but involvement of the liver dominated the clinical picture. Laboratory findings were conspicuous hypergammaglobulinemia, strongly positive flocculation reactions and increased erythrocyte sedimentation rate. Liver biopsies showed a picture similar to postnecrotic cirrhosis with infiltration of lymphocytes and plasma cells. They suggested the term *lupoid hepatitis* for this syndrome, which seems to be a characteristic entity to be distinguished from S. L. E. and from postnecrotic cirrhosis or chronic active hepatitis.

A patient with this syndrome has been under our observation for more than 1 year.

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Case report

The patient is a 26-year-old female who previously has been in good health. There is no history of liver disease or exposure to infection from hepatitis. At routine examination in 1958 the erythrocyte sedimentation rate (ESR) was 20 mm/hour, and following influenza the ESR was 50 mm/hour in December 1959. From early February 1960 she complained of general malaise, and the ESR was 30 mm/hour. Jaundice was observed on March 20th.

On admission to hospital elsewhere on March 25th she was icteric, the liver was tender and enlarged to 2 fingers below the costal margin. Laboratory investigations showed icteric index 24, strongly increased thymol turbidity, ESR 77 mm/hour and bilirubin and urobilinogen in the urine. The serum protein value was 10.6 g per 100 ml, of which 4.8 g were gammaglobulin on electrophoresis. During the following 12 days she lost 3 kg weight, and the laboratory data remained essentially unchanged (fig. 1). Prednisone, 30

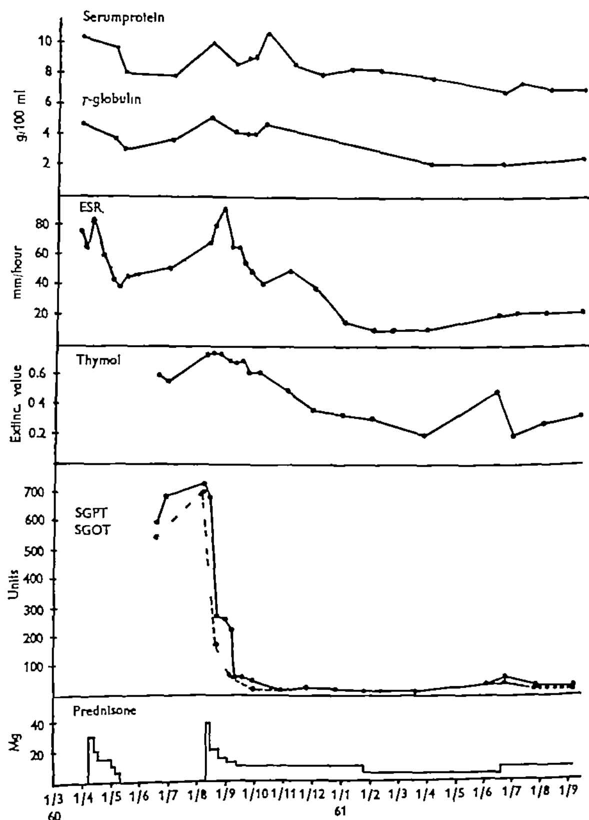


Fig. 1. Laboratory findings in relation to prednisone therapy in a patient with lupoid hepatitis.

mg orally was given on April 9th. The dose was gradually decreased, but prednisone therapy was continued until May 10th. Following this her general condition, as well as laboratory findings improved, and she was discharged on May 25th 1960.

When seen in the out-patient department at this hospital in July and August she complained of fatigue. In July she had a period with diarrhoea and later she complained of joint- and muscular pains. There had also been epistaxis. Thymol turbidity and ESR showed increasing values, the transaminase activity was very high, and admission to hospital was advised.

On admission to this hospital on August 9th 1960 she was slightly jaundiced. The liver was enlarged to 2 fingers below the costal margin, and the spleen was just palpable. There was facial acne, and a few spider naevi. The serum bilirubin concentration was 2.5 mg per 100 ml, and the urine contained urobilinogen and bilirubin. Thymol turbidity, ESR, serum proteins and transaminase activity are shown in fig. 1. Other findings were: Haemoglobin 13.1 g per 100 ml, white blood cell count 6,100 and platelet count 350,000 per mm, normal peripheral blood smear, alkaline phosphatase 7.5 Bodansky units, serum iron 170 γ per 100 ml, and the thrombotest reaction (11) 66 %. The p-toluenesulphonic acid precipitation test (7) was strongly positive (+++). Seroreactions for syphilis, cold agglutinins and antistreptolysin were negative, as well as the Waaler reaction and the Paul-Bunnel reaction. An X-ray of the chest was normal, and the electrocardiogram showed negative T-waves in leads V_{1-4}.

Shortly after admission she developed fever and severe pain in the left elbow which became red and swollen. This was followed by similar manifestations in other joints. Prednisone, 40 mg orally was given on August 13th followed by reduction of the dose to maintenance level of 10 mg daily. She became afebrile within a few days from onset of prednisone therapy, and fever and joint pains disappeared. There was a rapid fall in transaminase activity whereas the serum proteins, the hypergammaglobulinemia, ESR and thymol turbidity reaction showed slower response towards normalization (fig. 1). The thrombotest reaction remained at 60–94 throughout.



Fig. 2. LE-cell from patient with lupoid hepatitis.

Tests for LE-cells, while the patient was on prednisone therapy showed nucleophagocytosis on several occasions, and on 2 occasions LE-cells were found (fig. 2).

She was discharged on September 16th 1960 with a maintenance dose of prednisone of 10 mg which later was reduced to 5 mg.

At follow up examinations her general condition has remained good, and she has been able to work. At irregular intervals she has suffered attacks of severe abdominal pain of 1–2 days duration. She had similar unexplained attacks during both hospitalizations. In June 1961 the liver function tests showed some deterioration, but there was a good response to increase of prednisone (fig. 1).

She has now taken prednisone for more than one year without apparent side effects. Her condition is good, the liver and spleen are no longer palpable, and there has been no recurrence of joint pains. The liver function tests are however still definitely abnormal, indicating chronic liver disease.

Discussion

The clinical picture in this young female patient, with signs of severe prolonged liver disease and general symp-

toms such as arthritis, arthralgias and epistaxis, is similar to that described in patients with lupoid hepatitis (9 10 12) The diagnosis of lupoid hepatitis in this case is supported by the extreme hyper gammaglobulinaemia positive LE-cell test and response to steroid therapy

Lupoid hepatitis was originally defined as the combination of chronic active hepatitis with positive LE-cell phenomenon (9) When symptoms and signs common to both severe liver disease and S L. E. are present in a given case, it is difficult to decide whether the liver involvement is due to S L. E or the "lupoid manifestations are due to the liver disease Although hepatomegaly is quite common in S L. E. (2), histological lesions are slight and nonspecific (3) and have been termed "hepatic lupus (9) Altered serum proteins with a positive thymol turbidity reaction may be encountered in both liver disease and in S L. E Also, the p-toluenesulphonic acid precipitation test, originally introduced as a test for S L. E. (7) is frequently positive in infectious hepatitis (1) Hyper bilirubinaemia and highly increased transaminase activity must, however result from more severe liver involvement than "hepatic lupus On the other hand lupoid manifestations were not seen in a single case in a series of 310 patients with cirrhosis of the liver (3) Symptoms similar to S L. E. therefore seem to be rare in ordinary chronic liver disease.

A sufficient number of cases with liver disease and an S L. E. like syndrome have been reported in recent years to make a chance association of the two conditions in the same patient improbable. It seems more reasonable to consider these patients as suffering from a particular type of liver disease, and that lupoid hepatitis is an appropriate term for the condition. Others have also pointed out that young females with cirrhosis represent a particular group (4 13)

Mackay et al. have suggested that the manifestations of lupoid hepatitis may be due to an immunological mechanism (9 10 12) They postulate an abnormal reactivity of the antibody producing tissues, initiated by antigenic stimuli from hepatic tissue. They suggest that hepatocellular components may become antigenic through alteration following injury e. g by virus, or that liver cell damage releases antigenic material, which is normally inaccessible. Reaction of the antibodies with the liver and probably also other body tissues, causes further liver damage and may in some cases initiate S L. E. This selfperpetuating process they called *autoclasia*—"self-breaking-down The hypergammaglobulinaemia positive autoimmune complement fixation reactions (5) and response to steroid therapy were considered as evidence in favour of an immunological mechanism in this type of liver disease (10 12) A positive LE-cell test was regarded as indicating antinuclear factors, rather than the presence of classical S L. E. (12) Hypergammaglobulinaemia and a favourable response to steroid therapy is however also seen in post necrotic cirrhosis (6)

Although the syndrome of chronic liver disease with lupoid manifestations has been reported in a number of cases, not all of them have had positive LE-cell tests (4) This may be because the LE-cell phenomenon in lupoid hepatitis is often only weakly positive and may be difficult to elicit (12) It may therefore be questioned whether patients with liver disease should be separated into a particular group solely on the basis of a positive

LE-cell test. On the other hand, a positive LE-cell test gives the most direct evidence as to the presence of lupoid hepatitis, although the clinical symptoms probably are of equal importance.

Prognosis in untreated lupoid hepatitis is considered to be poor. Steroid treatment seems to be effective in controlling symptoms and may have to be continued indefinitely. Whether steroid therapy will affect the ultimate prognosis is at present unknown.

Summary

Lupoid hepatitis, i. e. chronic hepatitis with positive LE-cell phenomenon, is described in a 26-year-old female. Continuous prednisone therapy for more than one year seems to have been effective in keeping the condition under control without causing side effects. Steroid therapy may therefore have to be continued indefinitely.

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Malabsorption in Acrosclerosis

Disseminated scleroderma

By

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According to Ingram (1958) acrosclerosis differs basically from scleroderma (morphea) even though in the final stages both diseases may be so much alike that differentiation is sometimes almost impossible. Acrosclerosis is characterized by the primary localization of the lesions and their subsequent progression, and by the histological condition of the skin lesions. Systemic and visceral involvement are restricted almost exclusively to the syndromes defined as acrosclerosis.

We are aware of the fact that Ingram's classification is seldom referred to in the medical literature. We think, however that it is sufficiently useful to be adopted generally The lesions in the skin and internal organs of our patients were identical with those described in the medical literature as disseminated or systemic scleroderma.

The best known visceral manifestations of this condition are those of the digestive tract, the heart, the lungs and the kidneys. The radiological features of gastrointestinal involvement have in particular been frequently reported (Hale 1944 de Four 1951 Kemp Harper 1953 Abrams 1954 Boyd 1954 Lenwand 1954 Talbott 1956 van Staveren 1959) These radiological features (atonic distended oesophagus, locally dilated loops of the small bowel and sacculation in the colon, see figs. 1 2, and 3) are often so characteristic that in several of the reported cases acrosclerosis could be diagnosed before skin lesions had developed (Hale 1944 Abrams 1954 Piper 1955 Malkinson 1956 Miller 1959) In one of our patients we also made the diagnosis of acrosclerosis before skin lesions appeared.

Although there is an extensive literature on the anatomical (radiological) pathology of the intestinal tract in acrosclerosis, we have found only one report in which malabsorption was clearly demonstrated (Rosenthal 1957) Th present paper is a report of three successive cases of acrosclerosis with obvious malabsorption, seen in recent years.

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puncture. The fasting serum iron was 154 µg %, 1 hour after the oral iron load it was 216 µg %.

Other laboratory data are Haemoglobin, white blood count, differential blood count and bone marrow no abnormalities. In the bone marrow very little iron was demonstrable histologically. In the blood the LE phenomenon was negative. The urea clearance was 55 %, 2 years before it had been 75. The urine showed no abnormalities.

Owing to the severe course of the disease, with hectic fever we were forced to try corticosteroids in addition to cardiac therapy. The fever which had been present for two weeks, dropped sharply to normal. There was temporary subjective improvement. All absorption investigations were done after corticosteroids had been administered for about two weeks, so this therapy was not followed by rapid return to normal of the absorption defects. Two and a half months after the beginning of the treatment with corticosteroids the condition of the patient deteriorated rapidly and she died.

Case B. Female, aged 79. At the age of 64 she had noticed Raynaud phenomenon of the fingers. Later ulceration of the fingertip developed. Up to the age of 77 when a temporary anorexia and diarrhoea developed she enjoyed good health. She was admitted two years later because of anorexia and diarrhoea from which she had been suffering for some months. She was emaciated and pale.

On examination, heart and lungs were normal. Blood pressure was 160/60. There was distinct anaemia. The skin of hands and wrists was thickened and not pliable. Several fingertips showed scars of previous ulcerations. The mouth was small, the surface of the tongue smooth. There were no telangiectases in the face, no pigmentary changes or joint abnormalities. The physical examination was otherwise normal.

The laboratory data showed an iron-deficiency anaemia (Hb 5.8 g %, erythrocytes 3.45 mill/mm³, reticulocytes 12—24 /₀₀, plasma iron 30 µg). Bone marrow active erythropoiesis and no histologically demonstrable iron. There was no occult blood in the stools. The urine was normal. Urea clearance was 47 %. Radiological examination of the oesophagus showed a wide atonic oesophagus with some narrowing distally. There was no emptying in the recumbent position. Some jejunal loops of the small intestine were distinctly dilated, typical sacculation was present in the colon.

The patient withdrew from treatment. The iron deficiency was probably caused by intermittent loss of blood from the intestinal tract and not by an absorption defect, since oral iron treatment resulted in rapid improvement of the anaemia.

Case C. Female, aged 72. At the age of 70 a typical Raynaud phenomenon of both hands developed. Afterwards she became anorectic, complained of nausea and was admitted because of vomiting and considerable loss of weight. The stools were, apart from a short period of diarrhoea, quite normal.

On admission there was an obviously poor nutritional stat (weight 48 kg, length 162 cm). The skin was shiny with brownish pigmentation, especially in the folds of the palms of the hands and the leg joints. No pigmentation of the mucous membranes or scars were present. There were no skin lesions or ulcerations on the fingertips as found in acrosclerosis. The physical examination was otherwise normal. Blood pressure was 120/70, temperature was normal. Examination of Hb, white blood count, differential blood count, smear and urine gave normal results. The specific gravity of the urine was 1,023, the erythrocyte sedimentation rate 32/64. The pigmentation was not caused by an adrenal insufficiency because the excretion of 17 ketosteroids in the urine increased from 2.3 to 4.3 mg/24 hrs after stimulation with corticotropin.

Radiological examination of the oesophagus consistently showed an atonic, slightly dilated oesophagus which did not empty in the recumbent position. The passage of the barium in the small bowel was delayed and the jejunum showed considerably dilated coils. The stomach was normal. The colon showed the typical sacculation to a small extent. In tables I and II the results of the absorption investigations are presented.

With diet (temporarily by stomach tube) and an anabolic steroid we tried to improve the nutritional state but without success. Treatment with corticosteroids was continued for 3 months, at first orally and later par

Methods

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Case reports

Case A Female, aged 52 At the age of 38 she had noticed a typical Raynaud phenomenon of her hands. Later ulcerations of the finger tips and the typical skin lesions of acrosclerosis of hands, arms, and legs developed At the age of 52 she required admission because of progressive dyspnoea and oedema of the legs.

The history revealed no gastrointestinal or joint complaints. On admission, the typical lesions of acrosclerosis on hands, arms, legs and face were seen. The skin of the cheeks showed characteristic teleangiectasies. There were obvious signs of right ventricular heart failure. Blood pressure was 150/115 The heart was enlarged and without murmurs. At the bases of the lungs moist râles were heard. Body temperature varied between 38° to 40° C. There was no expectoration.

Radiologically the heart showed a general enlargement, which was not present on an X ray made two years earlier The hilar vessels were prominent, the lung fields clear The ECGs of the last two years showed a progressive right axis deviation, the most recent showed distinct right ventricular strain. These findings could be interpreted as an expression of pulmonary hypertension. Extensive pulmonary function tests, however showed no symptoms of diminished compliance of the lungs.

Radiological examination of the oesophagus showed an atonic oesophagus which failed to empty after half an hour in the recumbent position. The stomach appeared to be normal. In the small bowel a marked impairment of motility was present. Several loops, including distal ones, were dilated Radiological examination of the colon showed the typical sacculation. The results of the absorption investigations are presented in tables I and II

The investigation of iron absorption was not complete due to difficulties with vene-

puncture. The fasting serum iron was 154 μg %, 1 hour after the oral iron load it was 216 μg %.

Other laboratory data are Haemoglobin, white blood count, differential blood count and bone marrow no abnormalities. In the bone marrow very little iron was demonstrable histologically. In the blood the LE phenomenon was negative. The urea clearance was 55 %, 2 years before it had been 75 %. The urine showed no abnormalities.

Owing to the severe course of the disease with hectic fever we were forced to try corticosteroids in addition to cardiac therapy. The fever which had been present for two weeks, dropped sharply to normal. There was a temporary subjective improvement. All absorption investigations were done after corticosteroids had been administered for about two weeks, so this therapy was not followed by a rapid return to normal of the absorption defects. Two and half months after the beginning of the treatment with corticosteroids the condition of the patient deteriorated rapidly and she died.

Case B. Female, aged 79. At the age of 64 she had noticed Raynaud phenomenon of the fingers. Later ulceration of the fingertip developed. Up to the age of 77 when a temporary anorexia and diarrhoea developed she enjoyed good health. She was admitted two years later because of anorexia and diarrhoea from which she had been suffering for some months. She was emaciated and pale.

On examination, heart and lungs were normal. Blood pressure was 160/60. There was distinct anaemia. The skin of hands and wrists was thickened and not pliable. Several fingertips showed scars of previous ulcerations. The mouth was small, the surface of the tongue smooth. There were no telangiectases in the face, no pigmentary changes or joint abnormalities. The physical examination was otherwise normal.

The laboratory data showed an iron-deficiency anaemia (Hb 5.8 g %, erythrocytes 3.45 mill/mm³, reticulocytes 12—24 ‰, plasma iron 30 μg %). Bone marrow active erythropoiesis and no histological demonstrable iron. There was no occult blood in the stools. The urine was normal. Urea clearance was 47 %. Radiological examination of the oesophagus showed wide atonic oesophagus with some narrowing distally. There was no emptying in the recumbent position. Some jejunal loops of the small intestine were distinctly dilated typical sacculation was present in the colon.

The patient withdrew from treatment. The iron deficiency was probably caused by intermittent loss of blood from the intestinal tract and not by an absorption defect, since oral iron treatment resulted in rapid improvement of the anaemia.

Case C. Female, aged 72. At the age of 70 typical Raynaud phenomenon of both hands developed. Afterwards she became anorectic, complained of nausea and was admitted because of vomiting and considerable loss of weight. The stools were apart from short period of diarrhoea, quite normal.

On admission there was an obviously poor nutritional state (weight 48 kg, length 162 cm). The skin was shiny with brownish pigmentation, especially in the folds of the palms of the hands and the big joints. No pigmentation of the mucous membranes or scars were present. There were no skin lesions or ulcerations on the fingertips as found in acrosclerosis. The physical examination was otherwise normal. Blood pressure was 120/70, temperature was normal. Examination of Hb, white blood count, differential blood count, smear and urine gave normal results. The specific gravity of the urine was 1,023 the erythrocyte sedimentation rate 32/64. The pigmentation was not caused by an adrenal insufficiency because the excretion of 17 ketosteroids in the urine increased from 2.3 to 4.3 mg/24 hrs after stimulation with corticotropin.

Radiological examination of the oesophagus consistently showed an atonic, slightly dilated oesophagus which did not empty in the recumbent position. The passage of the barium in the small bowel was delayed and the jejunum showed considerably dilated coils. The stomach was normal. The colon showed the typical sacculation to a small extent. In tables I and II the results of the absorption investigations are presented.

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Methods

A barium enema and an oral barium meal with follow-through, were done in the usual way X-rays of the oesophagus were made in the standing and recumbent positions. The latter exposure was made after the patient had been in the recumbent position for half an hour after the oesophagus had been filled with contrast. Fat absorption was tested in a four-day balance study (intake-excretion) Faecal marking was done with carmine and purified charcoal. The diet contained a fixed quantity of fat (varying from 50 to 80 g per day) Measurement of the faecal fat content was done by the method of v d. Kamer (1949) In normal individuals fat excretion varies from 1 to 6 % of the intake. We consider an excretion of over 10 of the intake pathologically elevated. Vitamin B_{12} absorption was studied by means of labelled vitamin B_{12} according to Schilling (1953) in the Haematology Department of this hospital (Head C. H W Leeksma, M D) In normal persons the lower limit of excretion of $CO^{60}B_{12}$ in the urine during the first 24 hours amounts to 13 % of the administered dose (0 5 μC and 0.5 μg $CO^{60}B_{12}$) In case of lowered excretion the investigation was repeated with an intrinsic factor preparation of known potency Iron absorption was tested by measuring the serum iron level before and after ingestion of 176 mg iron in the form of ferrous chloride as indicated by Verloop (1958) The 24-hour calcium excretion in the urine was examined when the patient's diet contained 230 mg calcium. We consider an excretion of less than 50 mg/24 hrs pathologically low supporting a diagnosis of calcium malabsorption. The response to a water-load was tested according to Robinson—Power—Kepler (1941) Glucose tolerance tests were carried out in the usual way (oral load of 50 g glucose, normal carbohydrate intake during the preceding days) If the body weight differed markedly from normal the glucose load amounted to 0.8 g per kg body weight. Alkaline phosphatase was measured according to Bodansky (upper limit of normal 4 5 U) phosphorus according to Briggs (lower limit of normal 2 7 mg %) calcium according to Clark and Collip (normal values between 9 and 11 mg %) and carotene according to Engel (normal values over 70 μg %) Serum albumin was deduced from the measurement of the specific gravity of serum according to van Slijke and from paper electrophoresis. Fractional gastric analysis was carried out with an alcohol test meal and 0.5 mg histamine hydrochloride if necessary

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Fig. 1 Case C. Oesophagus. Half an hour film in recumbent position. Dilatation. No peristalsis.



Fig. 2. Case A. Gastrointestinal series. One hour film. Dilatation of jejunal loop.



Fig. 3 Case A. Barium enema. Sacculation of the transverse colon.

Our investigations show that absorption defects in acrosclerosis may be severe and may involve the distal part of the small bowel. We found that treatment with corticosteroids gave no rapid return to normal of the absorption defect (cases A and C).

In the literature the occurrence of malabsorption in acrosclerosis is rarely mentioned and incompletely investigated. Only Rosenthal gives convincing data (fat balance technique) on steatorrhoea in acrosclerosis. Cooke (1952) and Volwiller (1957) mention the occurrence of steatorrhoea as an established fact. Some of the patients reported by Skouby (1950) and Malkinson (1956) probably suffered from steatorrhoea. Cornbleet's data (1957) suggest that some of his patients had an impairment of calcium absorption. Flat oral glucose tolerance tests have been reported by Robles Gil (1951) and Abrams (1954) among others.

It is striking that, in contrast to the limited literature on malabsorption, the radiological changes in acrosclerosis are frequently reported. In our opinion there is no reason to conclude from this contrast that absorption disturbances are

Table I. Results of absorption tests and the effect of corticosteroids

	Patient A	Patient B	Patient C
Fat excretion as % intake	12	36	12
Iron absorption	Normal	Normal	Disturbed
Vit. B_{12} absorption (Schilling test)	Disturbed (7.26 %, with intrinsic factor 8.3 %)	Normal (14.11 %)	—
Urine-excretion calcium (oral load 230 mg)	44 mg/24 hr	11 mg/24 hr	—
Response to a water load (R.P.K.)	Disturbed	Disturbed	Normal
Oral glucose tolerance test	Normal	Normal	Normal
Effect of corticosteroids on fat-absorption	None	—	None

Table II. Some other data relevant to the function of the intestinal tract and the characteristic radiological changes

	Patient A	Patient B	Patient C
Serum calcium (mg/100 ml)	8.5	8.7	10.2
Serum alkaline phosphatase (Bodansky)	4.0	7.6	1.2
Serum inorganic phosphorus (mg/100 ml)	3.9	2.4	3.5
Serum carotene (μg/100 ml)	34	16	35
Serum albumen (g/100 ml)	4.5	4	3.4
Fractional gastric analysis	Histamine fast anacidity	No free acid	Histamine fast anacidity
Radiological changes:			
Oesophagus	+	+	+
Small bowel	+	+	+
Colon	+	+	+

enterally. At the end of this period the fat absorption was still disturbed to the same extent (18 % excretion) and the body weight had diminished to 40 kg. Gluten free diet had no effect on the nutritional state.

Results and comments

The results of our investigations are compiled in tables I and II.

In our three patients the absorption studies confirmed an impaired fat absorption. The indications of impaired calcium absorption found in two of the three patients probably have some relation to this. In patient B suspicion of beginning osteomalacia seems justified.

In one of the two patients investigated, vitamin B_{12} absorption which takes place in the distal part of the ileum, was subnormal. This defect was not caused by lack of intrinsic factor. To the best of our knowledge, impaired vitamin B_{12} absorption in acrosclerosis has not been previously reported.

The results obtained with iron and water loading may point to impaired absorption of iron and water, but an alternate interpretation of the data is possible.



Fig. 1 Case C. Oesophagus. Half an hour film in recumbent position. Dilatation. No peristalsis.



Fig. 2. Case A. Gastrointestinal series. One hour film. Dilatation of jejunal loop.



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rare The infrequency of reports on malabsorption in acrosclerosis may perhaps be explained by the circumstance that even in severe malabsorption the appearance of the stools may be rather normal. In our patients the stools were not obviously abnormal during the period of examination Pat. B produced nearly normal stools during a fat "excretion of 36 % This phenomenon has been mentioned in other forms of steatorrhoea by Cooke (1946)

It is our impression that hitherto in most cases of acrosclerosis malabsorption has not been sufficiently considered It is to be expected that these disturbances could be found more often if an investigation for malabsorption were instituted in every case of acrosclerosis

Summary

Three female patients suffering from acrosclerosis are presented Apart from the typical radiological features in oesophagus and small and large bowel we found distinct malabsorption All three patients showed a fat absorption defect as demonstrated by fat balance technique In two of the patients indirect evidence of a disturbance in calcium absorption was present One patient had a vitamin B_{12} absorption defect. Two patients were treated with corticosteroids without any amelioration in the absorption of fat The authors point to the fact that malabsorption in acrosclerosis very probably occurs more frequently than the relatively rare reports on this subject suggest They recommend an investigation for malabsorption in all patients suffering from acrosclerosis even if the stools seem to be normal.

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Cardiac Infarct and Weekend

By

Oleg Gorbatow Jorma Haavisto and Martti J Karvonen

The epidemiology of coronary heart disease has been the subject of many studies during recent years. All these investigations appear to show that the disease has become considerably more frequent over the last thirty years. However in several details the results of studies made in different countries show differences, evidently depending on the population and its mode of life. This applies also to such studies, in which attention has been paid to seasonal variations in the incidence of cardiac infarct. In Sweden, Finland and Germany infarction has been reported to occur most frequently in the winter months (Ekvall 1955 Doczauer and Naeve 1956, Vartio 1960 Gorbatow 1961), but also peak in September has been observed (Knuutti and Sydänmaa 1960). In the northern United States the frequency of infarcts is at its highest during the winter months (*e. g.* Bean and Mills 1938) but in the southern states a slight increase has been observed in the summer (Yater et al. 1948). Schnur (1956) however reports maximum during winter also in Houston, Texas.

Variation in the incidence according to the day of the week has also been reported. In 1939 Hallerman showed, using as his material the forensic autopsies in Berlin, that cardiac infarcts were more common during the weekend including Monday than during the rest of the week. Doczauer and Naeve (1956, 1959) studied a series of 5,256 coronary deaths among the forensic autopsies performed in Hamburg and came to a similar conclusion. An increase of cases at the weekend may also be seen in clinical material, as shown by Berg et al. (1957): their study covered 134 infarcts over 7 years in a Hamburg medical clinic. The distribution of their cases over the week is shown in fig. 1. Jensen (1959) has reported a similar study from Norway: 105 cases over the period 1953—56. A slight tendency to an increase at the weekend was observed, but it did not attain statistical significance. In Vartio (1960) series of 400 infarcts in Oulu, Finland, the incidence was significantly higher from Saturday to Monday than during the other weekdays.

The mode of life, and particularly that of spending the weekend may vary ap-

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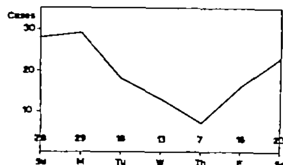


Fig 1 The distribution of cardiac infarcts to each weekday in a German clinical series (Berg et al 1957)

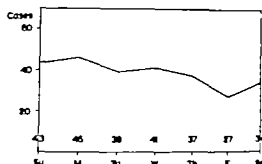


Fig 2 The distribution of cardiac infarcts to each weekday in the present Finnish series.

preciably from country to country and also among different population groups. Additional information on the distribution of cardiac infarcts over the week was considered particularly interesting for Finland because the majority of Finns take a hot bath ("sauna") every Saturday night A study of this problem was therefore made on the patients of two hospitals.

Material and methods

The material consists of 167 cases of cardiac infarct treated in the Maria Hospital in Helsinki during the year 1960 and of 107 cases of the Hyvinkää Hospital from the period 1959—60 In the Hyvinkää series, 22 cases came from a rural area and the remaining 85 from a small town. All cases with uncertain diagnosis, cases of sudden death, and infarcts which had occurred in connection with other illness were excluded. In addition to the clinical picture, the diagnosis was based on unipolar electrocardiography the usual laboratory tests (*e g* leukocyte count) and often also on the transaminase test.

The results were tested with the aid of the chi square method.

Results

The distribution of the cardiac infarcts in the entire series according to each day of the week is shown in fig 2. The variation is slight, and without statistical significance

In a study of the weekend it is appropriate also to include with the Sundays, public holidays such as Christmas, etc. Correspondingly the preceding day must be considered as equivalent to Saturday and the subsequent day to Monday Monday was included in the weekend in order to make the results comparable with those of previous investigators. In table I the cases on Saturday Sunday and Monday and similarly for the public holidays were pooled and compared with their expected number This was calculated with the aid of the calendar of 1959 and 1960 by dividing the infarcts to "weekend days" and "other days" in the proportion of such days. The slight excess of "weekend" infarcts is not significant

Table II presents a similar calculation, with the difference that only Saturdays and Sundays and the corresponding public holidays were considered as "weekend" thus excluding the Mondays. In table II the number of observed "weekend" infarcts is practically identical with the expected number The same conclusion applies to both Helsinki and Hyvinkää.

Since the program of the weekend is generally rather different for men and

Table I. The expected and observed number of cardiac infarcts during the weekend (Saturday, Sunday, Monday) and on other days, according to locality

Period	Helsinki			Hyvinkää			Total		
	Exp.	Obs.	Diff.	Exp.	Obs.	Diff.	Exp.	Obs.	Diff.
Weekend	76	80	4	50	57	7	126	137	11
Other days	86	82	−4	56	49	−7	142	131	−11
Total	162	162	—	106	106	—	268	268	—

$\chi^2 = 0.406$ $P > 0.05$

$\chi^2 = 1.835$ $P > 0.05$

$\chi^2 = 1.812$ $P > 0.05$

Table II. The expected and observed number of cardiac infarcts during the weekend (Saturday, Sunday) and on other days, according to locality

Period	Helsinki			Hyvinkää			Total		
	Exp.	Obs.	Diff.	Exp.	Obs.	Diff.	Exp.	Obs.	Diff.
Weekend	52	52	—	34	35	1	86	87	1
Other days	110	110	—	72	71	−1	182	181	−1
Total	162	162	—	106	106	—	268	268	—

Table III. The number and percentage of weekend (Saturday, Sunday, Monday) infarcts in women and men

Period	Women		Men		Total	
	Cases		Cases	%	Cases	%
Weekend	39	46	98	53	137	51
Other days	45	54	86	47	131	49
Total	84	100	184	100	268	100

$\chi^2 = 1.110$
$P > 0.05$

women, the sexes are treated separately in table III. In this table, Monday is included in the weekend. The tendency of men to get a weekend infarct is slightly higher than that of the women, but the difference does not attain statistical significance.

With advancing age, the mode of life may become more uniform, with less difference between the days of the week. Because of this assumption, the distribution of the infarcts to the weekend was studied separately for two age groups, drawing the limit at 60 years (table IV).

Table IV The number and percentage of weekend (Saturday Sunday Monday) infarcts before and after the age of 60 years

Period	Age				Total	
	—59		60—			
	Cases	%	Cases		Cases	%
Weekend	71	53	66	49	137	51
Other days	63	47	68	51	131	49
Total	134	100	134	100	268	100

$X^2 = 0.538$
$P > 0.05$

Table V The number and percentage of weekend (Saturday Sunday Monday) infarcts in the social classes I—II and III—IV

Period	Social class				Total	
	I—II		III—IV			
	Cases	%	Cases	%	Cases	
Weekend	47	48	84	53	131	51
Other days	50	52	74	47	124	49
Total	97	100	158	100	255	100

$X^2 = 0.599$
$P > 0.05$
13 cases could not be classified as to social classes.

The distribution is practically identical before and after 60

The social class may also affect the weekend customs. The patients were divided in four social classes, following directions given by the Helsinki City Statistical Office. Pensioners were classified according to the previous occupation married women without own occupation according to that of the husband. The higher social classes I and II were pooled similarly the lower ones III and IV The results are shown in table V The slight excess of weekend infarcts among the lower social classes was not significant.

Discussion

No accumulation of cardiac infarcts to the weekend was observed in the present series of city (167) and small town or rural (107) cases This result is in contrast with those of studies from Germany and Norway and with the study of Vartio from northern Finland according to which the frequency of infarcts appeared to be higher during the weekend (Saturday Sunday Monday) than during the rest of the week. No obvious explanation for this discrepancy may be suggested A large majority of the Finns take a hot bath (sauna) every Saturday whereas this custom is only sporadic in other

countries. The absence of a weekend peak in the incidence of cardiac infarcts in the present series is particularly noteworthy as the Saturday hot bath may be considered as a stress for the circulatory system, and hence an accumulation of infarcts at the weekend might well be expected.

Further active leisure time pursuits such as skiing, hiking, gardening, fishing and hunting are fairly common in the region studied, also among the city population. The German investigators Berg et al. consider the fat Sunday meal as an essential factor also in this respect, the populations studied may differ.

The material was divided into subgroups according to locality, sex, age, and social class. None of the subgroups showed a particular tendency for infarcts during the weekend.

Summary

A study was made of the distribution of the onset of 274 cases of cardiac infarct to each weekday. The differences between the weekdays were slight and statistically insignificant. There was no accumulation of infarcts at the weekend, neither in the whole material nor in subgroups divided according to locality, sex, age or social class. The result was the same, whether Monday was included in the weekend or not.

This result is in contrast to a weekend peak of infarcts as observed in some other studies. The absence of an accumulation of infarcts to the weekend is of particular interest in view of the fact that practically the entire population of Finland takes a hot bath ("sauna") every Saturday night.

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Restenosis of the Mitral Valve

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and Lars Werkö

After Bailey Harken, Smithy and Brock in 1948 had successfully performed digital valvotomy in mitral stenosis, the method came into general use. Different instrumental methods of operation were later introduced and the results have been good in 65—75 per cent of the cases, the condition unchanged in 15—25 per cent, and the operative mortality less than 10 per cent.

A successful mitral valvotomy should open up the valve to two fingers, open the commissures to the fibrous ring and free the chordae tendineae so that the cusps do not remain in apposition Pulmonary wedge pressure, resting pulmonary artery pressure and pressure rise t work should be reduced to nearly normal. Even though marked improvements of this kind have often not been found the functional condition has often been considerably improved postoperatively Long-term results published in later years, however show a rather great frequency of recurrences, sometimes as great as 25—30 per cent.

Submitted for publication September 30, 1961

In this paper we describe 8 cases some selected because of long follow-up time, some because of interesting history Some reasons for bad long-term results, particularly restenosis, are also discussed. The high reoperative mortality rate in this selected material is not to be considered as representative.

Case reports

From a follow-up material of bout 150 cases operated on for mitral stenosis eight cases are described, seven of which have shown signs of restenosis. A summary of each case is given together with table of symptoms and signs. Heart catheterization results have been brought together in one table. In some cases there have been fever increased sedimentation rate[1] and/or increased C-reacti protein[2] values, antistreptolysin titre[3] bove 400 U and, in a few joint symptoms and leukocytosis. These findings possibly indicate rheumatic activity To support such diagnosis, some type of heart affection ought to be proved.

[1] Here denoted SR
[2] Here denoted CRP
[3] Here denoted AST

We have not been able to use the PQ time, either because the patients have had atrial fibrillation or because electrocardiograms have not been available from the time in question. Since in many cases the auscultatory heart findings remain unchanged after operation one cannot expect any diagnostic help from them. During periods of suspected rheumatic activity the heart volumes have, as shown in the diagrams, increased, which further indicates such activity. The dyspnoea is classed from 0 to + + + + corresponding roughly to the heart insufficiency classification of the American Heart Association. Other symptoms have, when convenient, been graded from 0 to + +. Venous congestion grade + usually indicates mild cervical stasis and mild hepatomegaly. + + indicates signs of tricuspid insufficiency with pulsating cervical veins and pronounced liver swelling sometimes with pathological liver function tests. The degree of pulmonary stasis has been estimated roentgenologically.

Case 1. Woman born 1919. Rheumatic fever was diagnosed in 1936, and then followed increasing fatigue and effort dyspnoea, further accentuated after pregnancy in 1944. In 1950 pressure values in the pulmonary circulation were double the normal, symptoms class II—III. At operation that year the mitral cusps were fibrous and hard and the ostium could be digitally widened from the width of a fountain pen to that of 1 1/2 index fingers. Considerable regress (to class I) followed; pressures in pulmonary circulation returned to normal. In 1952 she was bedridden the whole year with fever and joint pains. Until 1955 there were repeated periods of suspected rheumatic activity and impairment. In 1956 atrial fibrillation developed and further impairment followed. In 1958 the BMR was lowered by means of antithyroid drugs with reasonable effect. In 1959 pulmonary circulation pressures were three times those just after operation and the patient was gravely handicapped (class IV). Reoperation that year was performed under cortisone protection because of signs of rheumatic activity: leukocytosis, AST-increase to 1 600, sedimentation rate increase. The ostium, with grave fibrous stenosis, was digitally widened from the width of less than a finger tip to 1 1/2 fingers giving considerable improvement (class I—II). The atrial fibrillation was then regulated, and the status has remained unchanged during continuous Dicumarol and penicillin treatment. May 1961 sinus rhythm, class I.

Case 2. Woman born 1909 complained of growing pains during childhood. Heart incompensation symptoms began in 1938 and increased until 1950 when she had incipient pulmonary oedema and greatly increased pulmonary circulation pressures. At commissurotomy that year both commissures were found calcified with a slit like opening (0.5 × 3—4 cm). Only the anterior one could be split, giving an ostium 2 fingers wide, mildly insufficient. Troubles decreased from class III to class I. Except for some attacks of atrial fibrillation 1952—1954 she was in excellent condition until 1956. Then rheumatic activity appeared (joint pains, SR and AST increase to 800 U) and returned 1957. In 1958 there was sudden impairment, complete disablement and death. The mitral ostium was barely open for the tip of the little finger, the left atrium was filled with thrombotic material; there were thrombi in the right atrium too. Pulmonary infarctions.

Case 3. Man born 1907. As a child he had recurring attacks of tonsillitis but no known rheumatic fever. Effort dyspnoea and incipient attacks of pulmonary oedema began in 1945. The symptoms increased to class III in 1951 when a digital commissurotomy was performed. The ostium was slit like, its width about 1/2 cm. Around the ostium there was calcification and the chordae tendineae were greatly thickened. The ostium could be dilated to the width of 2 fingers. A slight incompetence of the valve increased at operation. The patient was essentially in class I until 1956 in spite of having atrial fibrillation, constant from 1955. Then there followed grave impairment (to class IV) with the same pulmonary wedge pressure value as before operation and deterioration of other catheterization values too. At reoperation in 1958 the mitral cusps were found totally calcified with a long slit like ostium and incompetence. Correction could not be done, but after treatment with I the patient's condition was improved and has, at regular controls, been found to be of class II—III.

Case 4. Man born 1912. As child he had several attacks of tonsillitis but no known rheumatic fever. A valvular lesion was detected in 1942, and in 1947 his heart started fibrillating and he got effort dyspnoea and palpitations. In 1948 he had a transient left-sided hemiplegia after cerebral embolus. After a peritonsillitis in 1951 he had joint pains and signs of an increasing heart incompensation. In 1952 signs of new emboli (aphasia and circulation disturbances in the left foot). The pulmonary artery pressure was moderately raised, the pulmonary wedge pressure greatly so. At digital valvotomy the mitral ostium was widened from less than a fingertip to the width of 1 1/2 fingers. The valvular cusps were rigid and the margins were rough. The condition was considerably improved. In 1953 he had recurring joint pains, fever, AST and SR-increases, but after treatment, including antibiotics, he was able to resume his work. The complaints gradually increased until 1958 when there was a rapid impairment of condition (class II—III) with signs tricuspid incompetence. During spring 1961 when there were signs of rheumatic activity the patient was wholly disabled. H was treated with diuretics and also with steroids and penicillin, and he improved. At instrumental commissurotomy the cusps were found to be calcified, uneven, and the ostium was highly stenosed, not letting fingertip through. It was dilated to the width of 2 1/2 fingers. After operation he improved considerably (to about class II).

Case 5. Man born 191.. No known streptococcus infection or rheumatic fever. Until 1953 he had recurring attacks of bronchial asthma. Signs of heart incompensation started in 1948 and in 1949, after sinus rhythm had twice been restored, he got constant trial fibrillation. Before operation in 1953 the pulmonary circulation pressures were greatly raised and the patient was in class III—IV. After preoperative treatment with Dicumarol and penicillin the mitral valve, like cornet and calcified, was split, the ostium increasing in width from that of an index finger up to that of 1 1/2 fingers. The cusps were left rigid. The condition was good for 5 years (class I) but then it was impaired. In 1959 treatment with I^{131} (BMR decreasing to — 40 %) brought temporary improvement. Heart catheterization findings indicated increased resistance in the pulmonary circulation and mitral stenosis. Angiocardiography showed mitral restenosis and a mild incompetence of the valve. In view of his desperate condition commissurotomy was performed in 1961. The Dubost dilator was used and the ostium was widened from the width of one finger to that of two. The mitral incompetence, however, greatly increased, the left atrium dilated and the patient died t operation.

Case 6. Woman born 1912. As child diphtheria but no rheumatic fever. In 1944 mitral stenosis was diagnosed. Four years later trial fibrillation started, sinus rhythm was restored but the patient had increasing signs of heart incompensation (class II) and the pressures in the pulmonary circulation were raised. In 1953 digital commissurotomy was performed. The ostium, being fibrously stenosed to the width of an index finger tip, was dilated to thrice that width. Postoperatively there was a pericarditis without much fever. The condition was improved and remained reasonably good for about 6 years (about class I). Atrial fibrillation returned in 1959. It was temporarily reversed but symptoms of heart incompensation increased (to class II). She was treated with Dicumarol and penicillin. Angiocardiography and heart catheterization performed in 1961 indicated restenosis. The mitral ostium was open for the tip of the little finger and was widened t 2 fingers with the Belmont instrument. Early at operation ventricular fibrillation started. It could not be reversed and the patient died. The mitral cusps and chordae tendineae were thickened. There was also mild aortic stenosis.

Case 7. Woman born 1907. I 1921 she had rheumatic fever which later recurred several times. Signs of heart incompensation increased, culminating in pulmonary oedema attack in 1953. At digital commissurotomy the mitral ostium permitted only the tip of the little finger. At both commissures there were calcified noduli. The ostium was widened to 1 1/2 fingers. Postoperatively there followed pericarditis, fever, SR- and AST-increases and reversible atrial flutter-fibrillation. The operation gave little improvement and during 1954—1959 there was recurring rheumatic activity with fever, SR, CRP and AST

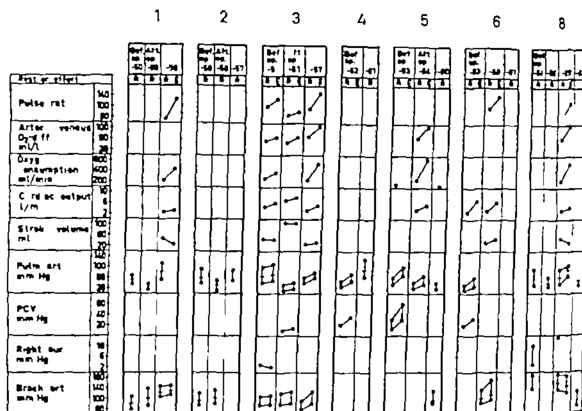


Fig 1 Heart catheterization values from patients nos. 1—6 and 8.

increases, and the patient was disabled (class IV) In 1958, lowering of the BMR with I¹³¹ had some effect but severe pneumonias made the patient moribund. In 1959 she got Prednisolone treatment and was reoperated on, with a digital commissurotomy There were tooth-like calcifications of the valve cusps and only the anterior commissure could be split, giving a 1 1/4 finger ostium with some incompetence. There were a period of pronounced heart incompensation and suspicions of a rheumatic relapse (increased SR and AST) but the result was a considerable improvement which remains (class II)

Case 8 Man born 1905 In 1928 he got rheumatic fever which later recurred several times. After 7 years he got effort dyspnoea and later attacks of pulmonary oedema, atrial fibrillation and peripheral emboli. Before operation in 1954 the symptoms had increased to class III and at heart catheterization pulmonary circulation pressures were found considerably raised. The mitral cusps were stone hard, giving a severe stenosis. Only the anterior commissure could be split, the posterior one being left untouched for fear of calcific emboli. The ostium was left 1 1/2 fingers wide, mildly incompetent. Postoperatively there was a pleural effusion, fever and SR increase. Then there was some improvement but after half a year bouts of impairment. In 1959 the patient was wholly disabled (class IV) At heart catheterization the pulmonary circulation pressures were about the same as preoperatively Valvotomy was done one year later but early in the operation there was heart arrest and the patient died. The mitral ostium was greatly stenosed and the cusp area thickened, like a cornet. Infarctions in the kidneys.

Comments

Before the first commissurotomy a streptococcus infection could be traced in five of the patients. Two of them gave histories of undoubted rheumatic fever

Table I

Case 1

	–36	–44	–50		–51	–52	–56	–58		–59			–59	–61
Rheum. activ	+			Digital commissurotomy		+			Treatm. with antithyroid drugs	+	Digital commissurotomy	+		
Atr fibrill.							+	+		+		+	—	—
Dyspnoea	+	++	+++		+	++	+++	++++		+++		+	+	+
Haemoptysis							+	+		+				
Venous stasis										++		+	+	+
Oedema							++	++		++		+	+	+
Periph. emboli												+		
Pulm. stasis			+			+	++	++		++		+	+(?)	+(?)
Tot. heart vol.			1,200			850				1,280		850		
Rel. heart vol.			680			520				770		530		

Case 2

		–38	–49	–50	–50	–52 54	–56	–57	–58	
Rheum. activ	+ (?)			Digital commissurotomy			+	+	+	Death
Atr fibrill.		+ (?)				+—+		+—	+	
Dyspnoea		+	++				++	+++	++++	
Haemoptysis										
Venous stasis								++	++	
Oedema			+					+	+	
Periph. emboli									+	
Pulm. stasis			+				+	+		
Tot. heart vol.			870		760	760	850	1,050		
Rel. heart vol.			630		500	500	600	690		

Case 3

		–45	–47	–49	–51	–53	–53–55	–55	–57	–58	–58	–58	–58	–61
Rheum. activ	+?				Digital commissurotomy						Resp. marked insufficiency; commissurotomy impossible	Treatment with I^{131}		
Atr fibrill.							+—+	+	+	+			+	+
Dyspnoea		+	++	+++		(+)	+	+	+++	++++			++	+++
Haemoptysis				+										
Venous stasis														
Oedema														
Periph. emboli														
Pulm. stasis		++	++	++				+	++	++			++	++
Tot. heart vol.				1,330		1,050		960	1 150				1,330	
Rel. heart vol.			620	700		550		540	630				780	

cm³ per m² body surface area.

Table I (cont.)

Case 4

	—42		—47	—48	—51	—52	—52	—52	—53	—54	—58	—59	—60	—61	—61	—61
	In childhood tonsillitis	Auscultatory mitral stenosis					Digital commissurotomy								Instrumental commissurotomy	
Rheum. activ					+				+		+	+?	+?	+		
Atr fibrill.			+	+	+	+		+	+	+	+	+	+	+		+
Dyspnoea			+	+	+	++		+	+	++	++	+++	+++	++++		++
Haemoptysis																
Venous stasis												+	++	++		++
Oedema												+	+	+		
Periph. emboli				+		+										
Pulm. stasis				(+)	+	+				+	+	+	+	++		++
Tot. heart vol				1 150		1 150			1 400			1,520	1 770			[illegible]
Rel. heart vol.				610		610			760			830	980			[illegible]

Case 5

		—48	—49	—52	—53	—53	—53	—57	—58	—59	—59	—59	—60	—61	—61
						Digital commissurotomy					Treatment with I			Instrumental commissurotomy	Death
Rheum. activ	+?														
Atr fibrill.		+	+—	+	+		+	+	+	++		+	+		
Dyspnoea		++	++	+++	++++		+	+	+++	+++		++	+++		
Haemoptysis															
Venous stasis					++				+	+		+	+		
Oedema															
Periph. emboli			+				+								
Pulm. stasis					++					+		+	++		
Tot. heart vol.		1 050			1,340		1 100	950		1,510			1,690		
Rel. heart vol.		550			720		600	500		760			850		

Case 6

	—44	—48	—50	—53	—53	—53-58	—59	—61		
	Mitral valv disease diag				Digital commissurotomy				Instrumental commissurotomy	Death
Rheum. activ										
Atr fibrill.		+—					+—+	+		
Dyspnoea		+	++	++		+	+(+)	++		
Haemoptysis										
Venous stasis								+		
Oedema							+	+		
Periph. emboli										
Pulm. stasis.			+	+			++	+		
Tot. heart vol.			800	1 100			770	970		
Rel. heart vol.			470	650			470	570		

Table I. (cont.)

Case 7

	—21	—28–30	—52	—55	—53	—53	—54	—57	—58	—59		—60
Rheum. activ	+	+	+		Digital commissurotomy	+	+	+?	Treatment with I^{131}	+	Digital commissurotomy	+
Atr. fibrill.						+−	+—	+—		+		+
Dyspnoea	+	++	++	++		++	+++	++++		+++		++
Haemoptysis										+		
Venous stasis							+	++		++		++
Oedema												
Periph. emboli												
Pulm. stasis		?	?	++		++	++	++		++		+
Tot. heart vol.												
Rel. heart vol.								580		580		580

Case 8

	—28	—35	—43	—46	—50	—54	—54	—54	—56	—59	—60		
Rheum. activ	+					+	Digital commissurotomy	+?				Instrumental commissurotomy	Death
Atr. fibrill.					+	+		+	+	+	+		
Dyspnoea		+	++	++	+++	++++		++	++	+++	++++		
Haemoptysis				+							++		
Venous stasis								+	+	++	++		
Oedema					+	+				++	++		
Periph. emboli					+	−							
Pulm. stasis				++							+		
Tot. heart vol.					910	900			800	1 100			
Rel. heart vol.					480	500			450	630			

two suspicions of it. Signs of mitral stenosis appeared, as a rule, during the fourth decade (14—38 years). Three patients had atrial fibrillation before the first operation and also peripheral emboli. Two patients had haemoptyses.

The first operations were performed during the years 1950 to 1954 by means of digital commissurotomy after a symptom period of 14 years on the average. Cases 1—3 were operated on by Prof. C. Crafoord in Stockholm and belong to the first five cases operated on there. They have been described by Werkö et al. 1952. Cases 4—8 were operated on by Dr G. Pettersson in Gothenburg. In all cases there were pronounced constrictions of the mitral ostia. Cusp calcifications were mentioned in six cases.

The ostial width is difficult to state exactly, partly because of the palpation difficulties mentioned below, partly because of the lack of an appropriate measuring system. In case 3 where certain incompetence arose, one can assume that the splitting went as far as the fibrous ring. It did so in case 2 where only the anterior commissure was split to the fibrous

ring In those two cases the width of the ostium was said to have become 2 fingers, in the others about 1 1/2 fingers

Preoperatively five of the patients were in class II two in class III and one in class IV The operation caused an improvement in seven cases, placing them in classes 0—I The most pronounced improvement of condition was in cases 1 2, 3 and 5 and in these cases the heart catheterization values indicated a favourable result In case 3 the pulmonary artery and wedge pressures regressed to normal and the stroke volume increased from 40 to 100 cm³ and in the others nearly normal values were reached The decrease in roentgenologic heart volume was also most pronounced in these cases. Here however there were postoperative mitral incompetences too.

The postoperative improvement lasted for 2 years in three of the patients for 4 years in one and for 6 years in three The duration of improvement seems to correlate with the completeness of the operation the aforementioned patients 1 2 3 and 5 being improved for 4 1/2 years in the average, the others (except for that one not improved at all) for 3 1/2 years

When the impairment had begun the symptoms as a rule progressed relatively fast as well as the roentgenologic heart volume and the pulmonary circulation pressures Atrial fibrillation appeared even in those patients who had preoperatively had sinus rhythm but in one of them sinus rhythm was restored, still persisting after 2 years.

In cases 1 3 5 and 7 the BMR was lowered by means of I¹ ¹ or antithyroid drugs, apparently with at least a transient effect corresponding to about one stage in the classification

One to seven years (on the average 4 years) after the appearance of the impairments 7 patients were reoperated[1] One patient died before reoperation could be managed Two patients then were in class II one in class III and five in class IV Increased resting pulmonary artery pressures and/or pulmonary wedge pressures and moreover lowered stroke volumes were found in all those patients of classes III and IV who were catheterized. Three of the seven patients reoperated on died two of them at an early stage of the operation one from heart arrest, one from ventricular fibrillation, the third owing to operative mitral incompetence. The mortality rate at reoperation then, was high in these cases, considerably higher than that stated by Belcher namely 8 % compared to 5.5 % at the first operation. One of our patients at the operation was found to have a combined mitral lesion which could not be corrected

In all cases but one (six operated patients and one post mortem case) the width of the ostium had decreased about one finger since the first operation. In the three cases where reoperation was successful the ostia could be dilated to between 1 1/4 to 2 1/2 fingers, and these patients were distinctly improved. They are now after a period of observation from 6 weeks to 2 years, in classes I—II

Discussion

The term restenosis is used by some only in cases where the first operation has been ideal, Bailey and Goldberg (1957) Patterson and Marshall (1959)

Belcher (1958 1960) and others use the terms true restenosis when at least one commissure has been fractured to the fibrous ring and false restenosis

[1] The reoperations were performed by Dr N P Bergh, Dr S. Ekestrom, Dr E. Linder and Dr C Pettersson

when the cusps have been left more or less in apposition. At digital commissurotomy with palpation through the left atrial appendage it is commonly difficult to localize the cusps and to check the completeness of the fracture. The distinction may therefore be hard to make.

In a five year follow-up of 42 cases, Glover et al. (1955) found no cases of restenosis. Their opinion like that of Brock (1952) is that restenosis after an ideal valvotomy is improbable unless the patient has a recurrence of rheumatic valvulitis. This is supported by Keyes and Lam (1954), McKusick (1955), Glenn and Dineen (1956).

Andros et al. (1953), Bailey and Goldberg (1957), Soulié et al. (1957), Ellis et al. (1958, 1959), Patterson and Marshall (1959) and Baker and Hancock (1960) have reported cases of restenosis after recurrent rheumatic activity. Among these patients there were also examples of true restenosis where signs of rheumatic activity could not be found. Baden (1958), Ross (1959) and Wilcken (1960) have not been able to demonstrate any rheumatic activity in clearly restenosed cases. Other reports, some concerning false restenosis, have been published by Jordan and Helkens (1952), Donzelot et al. (1953), Wood (1954), Baronofsky et al. (1953), Likoff and Urlecho (1958), Robb (1960).

The pathology of false restenosis has been discussed by Brock (1952) who focuses attention on the critical areas where the chordae tendineae insert on the cusps. If these critical areas have not been split agglutination goes on. Magarey (1951) however showed that the agglutination began peripherally in the commissures, and that this process in scarred commissure, could go on for a long time after the rheumatic inflammatory process had ceased.

Most of the reported cases are probably false restenosis. In a survey of 80 reoperations Harken et al. (1961) have found the following causes for bad postoperative results:

1. Inadequate initial surgical correction (45 per cent)
2. Mitral insufficiency preexisting or following operation (22 per cent)
3. Recurrence of rheumatic fever (17 per cent)

These figures will naturally vary in different series of patients. Bailey and Goldberg (1957) who followed 1 000 patients for up to 8 years found a 2.2 per cent frequency of "true" restenosis. The frequency of "true" and "false" restenosis has been 11 per cent in 294 patients followed by Belcher (1960) for one to seven years, in good agreement with Robb's (1960) figure of 11.8 per cent. Belcher has shown the frequency to increase with a longer follow-up time.

What, in our cases, have been the courses of the secondary deteriorations? In seven cases the primary operation has probably not been ideal, but the pressures in the pulmonary circulation were, however, lowered almost to normal in several cases, (cf. table I). False restenosis, then, cannot be excluded. In four cases, however, there is a strong evidence for reactivation of the rheumatic fever. There have been the previously mentioned findings, namely fever, rise in sedimentation rate, C-reactive protein titre, antistreptolysin titre above 400 U and in some cases joint pains, leukocytosis and signs of impaired heart function with increasing heart volume. In case 2 where the primary result was strikingly good and remained for six years, rheumatic activity seems to have been of great importance for the impairment. The same seems probable in cases 1 and 4. One patient (case 7)

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Serum β-Lipoprotein Lipids and Protein in Normal Subjects of Different Sex and Age

With a Note on the Separation of β-Lipoproteins by Chromatography on Hydroxylapatite

By

K. Cramér

It has been demonstrated that the levels of lipoproteins in human serum vary according to age and that some differences can be attributed to the sex of the patient (1—3). By way of summary these papers have indicated a rise in β-lipoproteins with increasing age — most marked in females during the menopause — and a higher level of α-lipoproteins in young females than in young males.

The determination of the composition of the lipoproteins in small materials has previously been published in a number of publications (4—9) and recently in larger normal materials and in different lipemic states (10—12). The cholesterol and phospholipid content of β-lipoprotein has been determined in a work by this author (13).

The protein moiety of the lipoproteins is difficult to determine after ultracentrifugation, as contamination by other serum proteins, especially albumin, has been difficult to avoid. Chromatography of serum on hydroxylapatite, however, as described by Hjertén (14) yields a highly purified product with negligible contamination (15) and makes it possible to achieve a quantitative isolation of the serum β-lipoproteins including the protein moiety. This investigation has been made in order to determine the concentration of the β-lipoprotein components in serum including the protein moiety as well as their relation to one another. It includes sera from normal persons of both sexes and at different ages.

Submitted for publication October 2, 1961

Clinical material

The material consisted of 15 men and 15 women, 20—40 years of age and 11 men and 11 women, 55—65 years of age. Each patient was interviewed to investigate the presence of suspected or definite atherosclerotic heart disease in the family as well as the incidence of juvenile diabetes or thyroid disease.

Aided by grants from the Swedish National Association for the Study of Heart and Lung Diseases and the Swedish Margarine Manufacturers Association.

who was not improved postoperatively showed signs of rheumatic fever reactivated immediately after operation with fever increases in sedimentation rate, antistreptolysin titre and pericarditis — that is the so-called post commissurotomy syndrome. Later signs of rheumatic activity recurred. Of the other cases (3 5 6 8) two showed signs of mitral insufficiency and one of aortic stenosis. The first had a mild, the others marked restenosis.

Summary

Restenosis of the mitral valve seems to be more usual than has hitherto been assumed the frequency rising with the time of observation. There are three causes of restenosis

1. False restenosis The first operation has not been satisfactory
2. "True" restenosis after a recurrence of rheumatic endocarditis.
3. "True restenosis where no rheumatic activity can be traced

Recurrences of rheumatic endocarditis are probably not as rare as was previously thought In eight patients from a follow up material of about 150 patients it is considered to have been of decisive importance in four cases The frequency of false and true restenosis is probably 11 to 12 per cent. Intense prophylaxis against streptococcus infections and penicillin treatment of all streptococcus infections are important. If a reactivation of rheumatic fever is suspected the patient ought to be treated as when rheumatic fever is proved The operation should if possible be postponed till the patient is symptomless. If there are suspicions of a restenosis, heart catheterization and angiocardiography usually have to be done to exclude a serious mitral incompetence or other lesions such as an aortic stenosis. Operative mortality is usually higher at the second operation even though figures as low as eight per cent have been given.

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Table I. Glyceride level of serum, β-lipoproteins (β L) and hydroxylapatite columns before and 4 hours after ingestion of 80 g butter fat

Subject	Fasting			4 hours after ingestion of 80 g butter fat		
	Serum	β-L	Column extract	Serum	β-L	Column extract
P. H.	0 65	0.37	—	1 17	0 44	—
K. C.	0.81	0 55	0.13	1.24	0.53	0.30
M. S.	0.96	0 50	0 07	1.85	0 48	0.78
B. C.	1.00	0.57	0 17	1 58	0 42	0.70
B. E. R.	1.26	1.53	0 14	4 50	1.80	0.75

The hydroxylapatite was dried after the elution with 0 65 M potassium phosphate buffer and extracted with chloroform methanol 1 1 ( ) The cholesterol and phospholipid values in these extracts were both less than 4 mg 100 ml serum in all cases except B. E. R., who had a mild hereditary hyperlipemia. In this case they were 24 and 41 mg 100 ml, respectively. The other subjects belong to the normal material, 20—40 years of age.

Table II. Isolated β-lipoprotein glycerides in patients with serum glycerides over 2.0 mMol/L

Serum glycerides mMol L	β-lipoprotein glycerides		Diagnosis
	mMol L	% of serum glycerides	
2.03	1 10	49	Xanthoma tendinosum
2 10	1 43	68	Survivor of myocardial infarction
2 62	2 13	81	Essential hyperglyceridemia
2 64	1 58	60	Estrogen treatment
2 75	2 65	96	Diabetic hyperglyceridemia
4 31	2 13	50	Essential hyperglyceridemia

added. The contents were mixed thoroughly and then centrifuged for 10 minutes at 2,000 r p m. 1 0 ml of the lower water-phase and

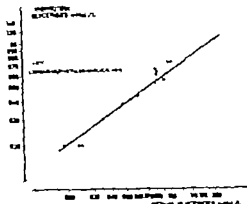


Fig. 1. Regression of β-lipoprotein glycerides on serum glycerides.
The calculations have been made on log 10 glyceride glycerol to avoid negative values.

2.0 ml of the upper hexanol-phase were taken to dryness at 110° and 160° C respectively. Determination of phosphorus in the residue was performed according to Svanborg and Svennerholm (17).

The water-phase contained the phosphorus of the phosphatidyl and phosphatidal compounds, for practical purposes equivalent to phosphatidyl phosphorus (22) and the hexanol-phase contained the phosphorus of the sphingolipids. The hydrolyses and phase partition were performed in duplicate and single determinations of phosphorus were carried out on each sample in a pair. The error of a single determination, calculated on 120 pairs, was 4.9 for the hexanol-phase and 3 5 for the water-phase.

Protein nitrogen was determined by a micro-Kjeldahl procedure, starting with wet combustion in concentrated H_2SO_4 in the presence of small amounts of cupric and mercuric ions. 30 perhydrol (Merck) was used when necessary. The specimen was transferred quantitatively to a Kirsten distillation apparatus, and 20 ml of 33 NaOH was added. Steam distillation was carried out for 6 minutes with 20.00 ml 0.010 N HCl in the recipient. The excess of HCl was titrated with 0.010 N NaOH, and methyl red/methylene blue was used as an indicator. Two blanks with 0.2 % EDTA were run simultaneously throughout the whole procedure.

The younger groups consisted of persons engaged in hospital work, i.e. laboratory workers, nurses, and medical students. They were not subjected to any further clinical investigation.

The older groups were taken from employees at the SKF ball bearing factory except for six of the women who were from the local Women's Autocar Corps. All were examined clinically including physical examination of heart and chest, blood pressure recording and measurement of hemoglobin and erythrocyte sedimentation rate. In addition, they performed an ECG exercise test on a bicycle ergometer. They performed a load of 900 kpm/min (for men) or 600 kpm/min (for women) without signs of coronary or pulmonary insufficiency.[1]

A diastolic pressure exceeding 95 mm Hg, a sedimentation rate exceeding 20 mm, and a hemoglobin content of less than 12 g/100 ml was regarded as abnormal.

Only those who were found to be healthy and who had a negative family history were accepted as normal persons. All kept an ordinary Swedish diet without any extra supply of unsaturated fat.

The sera were drawn in the fasting state.

Laboratory methods

β Lipoprotein isolation by chromatography on hydroxylapatite (14) as further described by Cramér and Bratteten (15). Columns with an internal diameter of 30 mm and with a filter G 1 were used. The average height of the sedimented hydroxylapatite column was 45 mm and the average weight of the dried hydroxylapatite 10 g. 0.2 of the disodium salt of etylenediamintetraacetate (EDTA) was added to all buffer solution and to the solution in which the hydroxylapatite was stored. Ten ml of undiluted serum was applied to the column. When the serum had entered the column, usually after one hour, 100 ml of 0.25-M potassium phosphate buffer solution of pH 6.8 was applied and run through the column. The flow rate was adjusted to an average of 10 ml per hour. It was observed that higher flow rates caused a less efficient separation and a lower recovery from the chromatography. It is especially important that the flow rate should be held low during the first few hours after the application of the 0.25-M phosphate buffer solution.

Under the above circumstances, the 0.25-M phosphate buffer eluate contained no traces of *β-lipoprotein* when tested immunologically by double diffusion in-gel precipitation according to Ouchterlony as described by Wadsworth (16).

A diffuse yellow zone was seen in the upper part of the column when the 0.25-M buffer solution had passed through. 0.65-M potassium phosphate buffer of pH 6.8 was then added. A well-defined, clearly orange-coloured front could be seen passing down the column. When this front was immediately above the filter of the column, the amount of buffer above the column was adjusted to about 15 ml and the final fraction collected in a separate test tube. Addition of more 0.65-M buffer gave no lipid-containing eluate. The preparation obtained was slightly opalescent and remained so also after prolonged standing.

Lipid extraction with chloroform/methanol 1/1 (v/v) was performed as described by Svanborg and Svennerholm (17). Repeated experiments verified that all inorganic phosphorus was recovered in the upper methanol-saline phase also when lipoproteins dissolved in 0.65-M phosphate buffers had been extracted.

Total cholesterol was determined according to Theorell as revised by Cramér and Isaksson (18). The error of a single determination was 1.08. The values have been reduced by 4.6 to make them comparable (18, 19) with values obtained by the method of Sperry and Webb (20).

Lipid phosphorus was determined according to Svanborg and Svennerholm (21). The conventional factor 25 was used to convert lipid P to phospholipid. The error of a single determination was 0.91.

Determination of hydrolysable and non-hydrolysable phospholipids was performed according to Svennerholm (22) after Robins et al. (23). An aliquot of the lipid extract, corresponding to 3—8 γ of phosphorus, was digested with 2.0 ml 0.50 N KOH at 38 C for 12 hours. 0.5 ml 6 N HCl was added and the contents mixed. After 1 hour 2.5 ml 1 hexanol was

[1] Thanks are due to Drs Gunnar Grimby and Harald Sanne of the Department of Clinical Physiology of the University of Goteborg, who performed the exercise tests.

different ages

β-Lipoprotein					
Cholesterol		Phospholipids		Glyceride glycerol mMol/L	Protein nitrogen mMol/L
mg/100 ml	mMol/L	mg/100 ml	mMol/L		
123 ± 9	3.19 ± 0.22	103 ± 7	1.33 ± 0.09	0.52 0.47–0.58 < 0.01	12.41 ± 0.87
173 ± 10	4.48 ± 0.26	125 ± 7	1.61 ± 0.09	0.56 0.49–0.63	13.89 ± 0.83
< 0.001		< 0.03			
101 ± 5	2.60 ± 0.12	88 ± 4	1.13 ± 0.05	0.32 0.28–0.36 < 0.01	10.44 ± 0.52
187 ± 11	4.83 ± 0.29	139 ± 9	1.79 ± 0.11	0.67 0.59–0.77	16.13 ± 0.86
< 0.001		< 0.001		< 0.001	< 0.001

The glyceride glycerol values are converted into logarithms before statistical calculation. The reconverted values of the mean for log glycerides ± the standard error of this mean are given in the table.

The values for β-lipoprotein cholesterol and phospholipid are given in mg/100 ml as well as in mMol/L.

P value as tested against the same age group in the other sex.
P value as tested against the younger age group in the same sex.

Table 1. Correlation coefficients for the β-lipoprotein components in normal males and females of different age groups

		rCN	rPN	rGN	rCP	rCG	rPG
Normal males	20—40 years = 15	0.84 0.001	0.86 < 0.001	0.53 0.05	0.93 < 0.001	0.35	0.53
	55—65 years 11	0.95 < 0.001	0.95 < 0.001	0.50	0.93 < 0.001	0.57	0.46
Normal females	20—40 years 15	0.68 < 0.01	0.77 < 0.001	0.48	0.78 < 0.001	0.50	0.53
	55—65 years 11	0.96 < 0.001	0.96 < 0.001	0.88 < 0.001	0.94 0.001	0.79 < 0.01	0.90 < 0.001

r = correlation coefficient. C = cholesterol. P = lipid phosphorus. G = log. glyceride glycerol. N = protein nitrogen.

The P values refer to t-tests for the significance of the r values with n − 2 degrees of freedom.

rCN = correlation coefficient between C and N etc.

Table III Concentration of serum lipids and of β-lipoprotein components in normal males and females at

		Serum		
		Cholesterol mg/100 ml	Phospholipids mg/100 ml	Glyceride glycerol mMol/L
Normal males	20—40 years	186 ± 8	209 ± 5	0.67 0.60—0.74
	15			< 0.01
	55—65 years	213 ± 9 < 0.001	236 ± 10 < 0.001	0.79 0.70—0.90
	11	< 0.05	< 0.02	
Normal females	20—40 years	175 ± 9	214 ± 11	0.41 0.36—0.47
	15			< 0.01
	55—65 years	261 ± 9 < 0.001	274 ± 8 < 0.001	0.96 0.87—1.07
	11	< 0.001	< 0.001	< 0.001

The values are given ± the standard error of the mean.

Table IV Molar relationships within the β-lipoproteins in normal males and females of different ages

		β Lipoprotein molar ratios				
		Ratio lipid/10 mol protein N			Cholesterol / Lipid P	Phosphatidyl P / Sphingolipid P
		Cholesterol	Lipid P	Glyceride glycerol		
Normal males	20—40 years	2.60 ± 0.12	1.09 ± 0.05	0.43 0.40—0.47	2.39 ± 0.06	2.37 ± 0.11
	15			< 0.05		
	55—65 years	3.24 ± 0.06 < 0.01	1.14 ± 0.03	0.39 0.35—0.43	2.80 ± 0.07	2.06 ± 0.17 < 0.02
	11	< 0.001			< 0.001	< 0.02
Normal females	20—40 years	2.53 ± 0.10	1.09 ± 0.03	0.31 0.28—0.34	2.32 ± 0.07	2.15 ± 0.11
	15			< 0.05		
	55—65 years	2.99 ± 0.05 < 0.01	1.11 ± 0.02	0.41 0.38—0.45	2.71 ± 0.05	2.36 ± 0.10 < 0.02
	11	< 0.001			< 0.001	

The values are given ± the standard error of the mean.

The glyceride/protein ratios were converted into logarithms before statistical calculation. The reconverted values of the mean for log glyceride/protein ± the standard error of this mean are given in the table.

P value as tested against the same age group in the other sex.
P value as tested against the younger age group in the same sex.

rPC.C	rCN.P	rGN.P	rCG.P	rCN.G	rPN.G	rCP.G
0.67	0.13	0.52	0.07	0.83	0.86	0.94
				< 0.001	< 0.001	< 0.001
− 0.10	0.58	− 0.50	0.49	0.95	0.95	0.93
				< 0.001	< 0.001	< 0.001
0.41	0.25	0.38	− 0.11	0.58	0.73	0.74
				< 0.05	< 0.01	0.01
− 0.13	0.60	0.17	0.76	0.68	0.81	0.85
			< 0.05	< 0.05	< 0.01	< 0.01

The P values refer to t-tests for the significance of the r values with n − 3 degrees of freedom. rCP = partial correlation coefficient between C and P keeping N constant etc.

The reader is referred to tables III—VI for detailed results. An outline of their principal features is given below.

Age differences

The serum lipids and β-lipoprotein lipids increased with age in both sexes. Exceptions were the serum glycerides and β-lipoprotein glycerides which increased only in females. The β-lipoprotein protein increased with age in females, but not significantly in males.

Of the β-lipoprotein molar ratios, the cholesterol/protein ratio increased with age in both sexes, while the lipid P/protein and glyceride/protein ratios did not change. The cholesterol/lipid P ratio increased with age in both sexes, while the phosphatidyl P/sphingolipid P ratio increased only in males.

Sex differences

Young females showed lower serum glyceride values than young males, but otherwise there were no different levels of serum lipids. Their β-lipoprotein cholesterol and glyceride values were lower than in young males.

The β-lipoprotein protein showed no significant sex differences.

In the older age groups, females had higher serum cholesterol and phospholipid values than males, but not higher serum glycerides. The β-lipoprotein lipids and protein did not significantly differ from one another. Of the β-lipoprotein molar ratios, the cholesterol/protein ratio was higher in males than in females, as was the phosphatidyl P/sphingolipid P ratio, while the other ratios were equal.

Correlation analysis

The correlations between cholesterol, lipid P and protein were significant in all pairs, while the correlations between glycerides and the other components were significant only in older females.

The partial correlations, keeping one variable constant, were significant when glyceride was kept constant, and only occasional significances were seen in the other coefficients.

Table VI Partial correlation coefficients for the β-lipoprotein components, keeping one variable constant

		$r_{CP.N}$	$r_{CG.N}$	$r_{PG.N}$	$r_{PN.C}$	$r_{GN.C}$
Normal males	20—40 years n = 15	0.85 < 0.001	− 0.22	− 0.37	0.35	0.67 < 0.05
	55—65 years n = 11	0.30	0.27	0.57	0.58	− 0.17
Normal females	20—40 years n = 15	0.55 < 0.05	0.26	− 0.07	0.52	0.22
	55—65 years n = 11	0.25	− 0.36	0.43	0.60	0.55

r = correlation coefficient. C = cholesterol. P = lipid phosphorus. G = log glyceride glycerol N = protein nitrogen.

The values were corrected for the phospholipid nitrogen, which was calculated as 0.45 x lipid phosphorus (4).

The error of a single determination was 0.13 %.

Glyceride glycerol was determined according to Carlson and Wadström (24) as simplified by Carlson (25). All values were expressed in molar concentrations.

The error of a single determination was 2.0 %.

The error of the chromatographic procedure + the lipid and protein determinations was calculated from 8 double determinations and found to be 5 % for cholesterol and glycerides, and 3 % for lipid P and protein N.

PRESENTATION OF RESULTS

The statistical calculations were carried out according to H. Cramér (26) and Kemp (26a). When presenting the relative proportions of the lipoprotein constituents, it was decided to refer the lipids to 10 moles of protein nitrogen, in order to put the lipid components in relation to the protein moiety. As a consequence all lipid concentrations had to be expressed in moles. This mode of presentation has been applied earlier for glycerides, and appears more satisfactory for phospholipids than the usual conversion of the lipid phosphorus value into phospholipid by the factor 25. The use of this factor necessitates an assumed fatty acid molecular weight and constant proportions between the main phospholipid fraction.

The concentrations of β-lipoprotein cholesterol and phospholipid in serum will be given in mMol/L and in mg/100 ml after conversion of lipid P into phospholipid by the factor 25.

The values for glyceride glycerol in serum and β-lipoproteins, as well as the ratios glyceride glycerol/protein nitrogen in the β-lipoproteins, showed a skew distribution as pointed out by Carlson (27). They have been converted into their corresponding logarithms, which were normally distributed. The mean value for the logarithms ± the standard error of the mean have then been reconverted into glyceride values.

Results

The isolated amount of glycerides from serum in relation to the serum glyceride level is shown in fig. 1. The correlation and regression coefficients were calculated on log 10 x glycerides to avoid negative values in the calculations. The amount shows a proportionately lower increase than the serum glyceride values. Test meals with 80 g butter fat in the form of cream had no influence on the glyceride values in the eluates after 4 hours as shown in table I. The isolated glycerides in six persons with serum glyceride levels exceeding 2.0 mMol/L are shown in table II.

$r_{PG.C}$	$r_{CN.P}$	$r_{GN.P}$	$r_{CG.P}$	$r_{PN.C}$	r_{PN}	$r_{CP.N}$
0.07	0.13	0.52	0.07	0.83 < 0.001	0.86 < 0.001	0.94 < 0.001
−0.18	0.58	−0.50	0.49	0.95 < 0.001	0.93 < 0.001	0.93 < 0.001
0.41	0.25	0.38	−0.11	0.58 < 0.05	0.73 < 0.01	0.74 < 0.01
−0.13	0.60	0.17	0.76 < 0.05	0.68 < 0.05	0.81 < 0.01	0.83 < 0.01

The P values refer to t-tests for the significance of the r values with n −3 degrees of freedom.
$r_{CP.N}$ = partial correlation coefficient between C and P keeping N constant etc.

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		$r_{CP \cdot N}$	$r_{CG \cdot N}$	$r_{PG \cdot N}$	$r_{PN \cdot C}$	$r_{GN \cdot C}$
Normal males	20—40 years n = 15	0.85 < 0.001	− 0.22	− 0.37	0.35	0.61 < 0.05
	55—65 years n = 11	0.30	0.27	0.57	0.58	− 0.17
Normal females	20—40 years n = 15	0.55 < 0.05	0.26	− 0.07	0.52	0.22
	55—65 years n = 11	0.25	− 0.36	0.43	0.60	0.55

r = correlation coefficient. C = cholesterol. P = lipid phosphorus. G = log glyceride glycerol
N = protein nitrogen

The values were corrected for the phospholipid nitrogen, which was calculated as 0.45 x lipid phosphorus (4)

The error of a single determination was 0.13

Glyceride glycerol was determined according to Carlson and Wadstrom (24) as simplified by Carlson (25) All values were expressed in molar concentrations.

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The error of the chromatographic procedure + the lipid and protein determinations was calculated from 8 double determinations and found to be 5 for cholesterol and glycerides, and 3 % for lipid P and protein N

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The statistical calculations were carried out according to H Cramér (26) and Kemp (26a) When presenting the relative proportions of the lipoprotein constituents, it was decided to refer the lipids to 10 moles of protein nitrogen, in order to put the lipid components in relation to the protein moiety As a consequence, all lipid concentrations had to be expressed in moles. This mode of presentation has been applied earlier for glycerides, and appears more satisfactory for phospholipids than the usual conversion of the lipid phosphorus value into phospholipid by the factor 25 The use of this factor necessitates an assumed fatty acid molecular weight and constant proportions between the main phospholipid fraction

The concentrations of β-lipoprotein cholesterol and phospholipid in serum will be given in mMol/L and in mg/100 ml after conversion of lipid P into phospholipid by the factor 25.

The values for glyceride glycerol in serum and β-lipoproteins, as well as the ratios glyceride glycerol/protein nitrogen in the β-lipoproteins, showed a skew distribution as pointed out by Carlson (27) They have been converted into their corresponding logarithms, which were normally distributed. The mean value for the logarithms ± the standard error of the mean have then been reconverted into glyceride values.

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The isolated amount of glycerides from serum in relation to the serum glyceride level is shown in fig 1 The correlation and regression coefficients were calculated on *log 10 x* glycerides to avoid negative values in the calculations. The amount shows a proportionately lower increase than the serum glyceride values. Test meals with 80 g butter fat in the form of cream had no influence on the glyceride values in the eluates after 4 hours as shown in table I The isolated glycerides in six persons with serum glyceride levels exceeding 2.0 mMol/L are shown in table II

the main vehicle for the transport of glycerides in fasting sera.

There is no sharp chemical or physical borderline between the lipoproteins with a density of less than 1 006 and the chylomicra. The chylomicra are often defined as lipoprotein complexes which cause serum turbidity (35 36). This fraction, however is far from homogenous. It can be divided electrophoretically into one fraction that migrates with the α_2 globulins and another with the *same mobility as the β-globulins* (37 38) if the chylomicra are isolated from patients with essential hyperglyceridemia.

Jobst and Schettler (36) demonstrated differences in composition between alimentary chylomicra and chylomicra in hyperglyceridemia. *This has been further* developed by Cornwell et al. (11) who defined parts of the hyperglyceridemic chylomicra as lipoproteins belonging to density class 0.96—1 006 but they were unable to achieve a more specific differentiation towards the alimentary chylomicra. They also established the presence of chylomicra in sera from normal fasting individuals. This fraction was defined as the top layer after centrifugation of serum at 9.500 g for 30 minutes with a solvent density of 1.006

There are several reports on the existence of α_1-lipoproteins in isolated chylomicra (39 40 41) and Furman et al. have demonstrated that ultracentrifugally typical α_1-lipoproteins can be isolated from lipoproteins in hyperglyceridemia with very low density (< 1 006)

It has also been possible to prepare specific antisera against chylomicra (42) which do not precipitate β-lipoproteins

It is thus clear that there exists a structural difference between chylomicra of alimentary origin and chylomicra" of lipoprotein character in hyperglyceridemia, but this difference is only partly explained.

When the chromatographic separation is applied to post prandial hyperglyceridemic sera, no increase in the recovered β-lipoprotein glyceride is seen, while the "trapped" glycerides in the columns increase. Sera from fasting hyperglyceridemic individuals, on the other hand are turbid and yield turbid preparations of β-lipoprotein with a high content of glycerides. This indicates that the alimentary chylomicra are trapped in the column, while the glyceride-rich, low density lipoproteins of hyperglyceridemia are eluated. The chromatographic separation differs in this respect from the procedure of Carlson (43) who found identical increases in serum and β-lipoprotein glycerides after a test meal.

The correlation between serum glycerides and lipoprotein glycerides in normal material is high, r = 0.77 which speaks in favour of a strong linear relationship between the two variables (fig 1)

The serum glycerides have been chosen as the independent variable in the regression analysis, but the dispersion observed in the individual values should be wider than the real dispersion because of the errors in the determinations. The range between the observed extreme values should therefore be somewhat wider than for the real values and the true regression coefficient somewhat higher than the value of 0.71 which was found. This deviation, however ought to be relatively small.

The regression line has a slope of 35 4 which means that the β-lipoprotein glycerides increase less than the serum glycerides. The percentage β-lipoprotein glyceride calculated from the regression line is 102 at a serum glyceride level of 0.20 mMol/L, 75 at 0.60 mMol/L, 65 at 1 00 mMol/L, and 43 at 1.50 mMol/L.

Discussion

Our present knowledge of the lipoproteins are based principally on investigations carried out in the ultracentrifuge. The lipoproteins are brought to flotation to the surface of a solution of well defined density or layered in a tube with a density gradient and their concentrations are measured optically in the analytical ultracentrifuge or chemically after lipid extraction of the layers of the tubes in the preparative ultracentrifuge.

Different solvent densities have been employed by different workers, and different classifications of flotation classes of lipoproteins have been established on the basis of work with the analytical ultracentrifuge. The vast literature in this field has recently been reviewed by Cornwell and Kruger (28)

The most commonly adopted method is that of Havel Eder and Bragdon (6) with the solvent densities modified to 1 006 1 019 and 1 063 for separation The density classes of lipoproteins their corresponding Svedberg flotation classes (Sf classes) in the analytical ultracentrifuge and their electrophoretic mobility on starch blocks are given below (29)

Density class	Sf class	Electrophoretic mobility on starch
0.96—1 006	20—400	
1 006—1 019	12—20	β
1 019—1 063	0—12	β

The fact that the majority of the low density lipoproteins migrate with the β-globulins in an electric field has made the term "β lipoprotein almost synonymous with low density lipoprotein i. e lipoproteins with a density of less than 1 063 This denomination is not consequent but has won wide-spread recognition (29 30 31)

The protein moiety of the β-lipoproteins decreases with decreasing density and shows structural differences in the different lipoprotein classes (32) These changes, however do not include the antigenic characters of the protein moieties which are similar over the whole range of lipoprotein complexes with densities of 0.96—1 063 (33 34)

The chromatographic method

The present method for isolation yields an immunologically homogenous product which has been shown (15) to contain lipoproteins with densities from 1.063 down to below 1 006 The isolation is based on the chromatographic behaviour of the lipoprotein on hydroxylapatite. Electrophoresis of the preparations has shown fractions of different mobility with 85 % lying in the region of the β-globulins, and the remaining 15 % migrating in front of these. The term β-lipoprotein has nevertheless been adopted for the whole preparation, and does not appear to be less appropriate here than when it is applied to preparations from the ultracentrifuge. It is to be understood as the fraction that is eluted from serum on hydroxylapatite columns with 0.65-M potassium phosphate buffer of pH 6.8 after elution with a 0.25-M buffer

It is essential to define the density classes that are contained within the preparations from hydroxylapatite columns. Much knowledge can be gained from a study of the glyceride recovery from serum. About 20 % of the serum glycerides in healthy persons are transported with the α-lipoproteins (29) and a varying proportion with the chylomicra, which are present only in small amounts in sera from fasting individuals. The β-lipoproteins on the other hand are

the main vehicle for the transport of glycerides in fasting sera.

There is no sharp chemical or physical borderline between the lipoproteins with a density of less than 1.006 and the chylomicra. The chylomicra are often defined as lipoprotein complexes which cause serum turbidity (35, 36). This fraction, however, is far from homogenous. It can be divided electrophoretically into one fraction that migrates with the α_2 globulins and another with the same mobility as the β-globulins (37, 38) if the chylomicra are isolated from patients with essential hyperglyceridemia.

Jobst and Schettler (36) demonstrated differences in composition between alimentary chylomicra and chylomicra in hyperglyceridemia. This has been further developed by Cornwell et al. (11) who defined parts of the hyperglyceridemic chylomicra as lipoproteins belonging to density class 0.96—1.006, but they were unable to achieve a more specific differentiation towards the alimentary chylomicra. They also established the presence of chylomicra in sera from normal fasting individuals. This fraction was defined as the top layer after centrifugation of serum at 9.300 g for 30 minutes with a solvent density of 1.003.

There are several reports on the existence of α_1-lipoproteins in isolated chylomicra (39, 40, 41) and Furman et al. have demonstrated that ultracentrifugally typical α_1-lipoproteins can be isolated from lipoproteins in hyperglyceridemia with very low density (< 1.006).

It has also been possible to prepare specific antisera against chylomicra (42) which do not precipitate β-lipoproteins.

It is thus clear that there exists a structural difference between chylomicra of alimentary origin and chylomicra of lipoprotein character in hyperglyceridemia, but this difference is only partly explained.

When the chromatographic separation is applied to post prandial hyperglyceridemic sera, no increase in the recovered β-lipoprotein glyceride is seen, while the "trapped" glycerides in the columns increase. Sera from fasting hyperglyceridemic individuals, on the other hand, are turbid and yield turbid preparations of β-lipoprotein with a high content of glycerides. This indicates that the alimentary chylomicra are trapped in the column, while the glyceride rich, low density lipoproteins of hyperglyceridemia are eluated. The chromatographic separation differs in this respect from the procedure of Carlson (43) who found identical increases in serum and β-lipoprotein glycerides after a test meal.

The correlation between serum glycerides and lipoprotein glycerides in normal material is high, r = 0.77, which speaks in favour of a strong linear relationship between the two variables (fig. 1).

The serum glycerides have been chosen as the independent variable in the regression analysis, but the dispersion observed in the individual values should be wider than the real dispersion because of the errors in the determinations. The range between the observed extreme values should therefore be somewhat wider than for the real values, and the true regression coefficient somewhat higher than the value of 0.71 which was found. This deviation, however, ought to be relatively small.

The regression line has a slope of 35.4 which means that the β-lipoprotein glycerides increase less than the serum glycerides. The percentage β-lipoprotein glyceride calculated from the regression line is 102 at a serum glyceride level of 0.20 mMol/L, 73 at 0.60 mMol/L, 65 at 1.00 mMol/L, and 43 at 1.50 mMol/L.

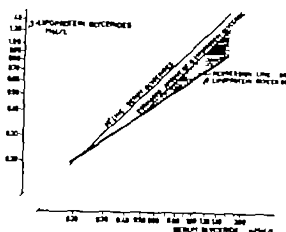


Fig 2 Schematic representation of the glycerides not included in preparations from hydroxylapatite columns.

The found regression line for the β-lipoprotein glycerides is drawn with a double line.

The left 45 lin represents th serum glycerides. The space between this l ne and the right 45 line represents the estimated amount of α-lipoprotein glyceride, calculated as 20 % of the serum glycerides. This percentage has been considered constant thro gh the range of serum glyceride values.

The hatched area between the α" area and the regression line represents non-isolated β-lipoprotein glycerides or chylomicron glycerides

Fig 2 shows a schematic representation of the composition of the non-β-lipoprotein glycerides. The construction of the diagram is given in the legend The α lipoprotein glycerides occupy the whole space between the regression line and the 45 line of 100 / recovery at the lowest glyceride values, but an increasing area is left between the α lipoprotein glyceride space and the regression line when the serum glycerides increase The estimation of the α-lipoprotein glyceride is approximate and may be erroneous, especially at the higher levels for serum glycerides. It seems probable, however that an increasing amount of β-lipoprotein or chylomicron glycerides is lost with increasing serum glycerides although it is not possible to calcul this amount exactly Judging from t data on post prandial and hyperglyc idemic sera the glyceride loss wou indicate the presence of chylomicra the alimentary type also in sera fr fasting individuals It is impossible establish to which extent the glycer loss may be incorporated in the lip proteins with densities of < 1 006.

Serum lipids

The serum cholesterol and phospholip alues in the present normal material a within the range of values presented fro the same region of Sweden (the west coa by Svanborg and Svennerholm (21) an Malmcrona (44) but lower than those Carlson (27) from Stockholm on the ea coast. A multitude of factors, however hav influence on the serum lipid values, such a dietary habits (29) seasonal variations (4 environmental factors, and "stress (46, 47, All of these have not been taken into con sideration in these materials. Moreover th increase in serum lipids is not continuou with age (48) and different age intervals o uneven rise have been found during investi gations (49) The selection of narrow age limits for the normal groups may therefor lead to misleading results. Comparisons be tween different investigations seem justified only if the investigations are carefully co ordinated and carried out simultaneously

The serum glyceride values most likely are subject to the same influences as the cholesterol and phospholipid values. Those found here are lower than those reported by Carlson (27) Svanborg and Svennerholm (21) and Björntorp (50) who all applied the same method of determination, and also lower than those found by Furman et al. (12) who used the method of van Handel and Zilversmit (51) It is especially apparent that the young females show very lo values for serum glycerides down to 0.20 mMol/L. Lower values for young females than for

young males have also been reported by Forman et al. (12) with a significant increase with age, similar to that found in the present material. Their normal male material covers patients 12—55 years of age and can not be compared with the present one. Other figures on serum glycerides with direct determinations have not been published so far.

β-Lipoprotein components

The changes within the β-lipoprotein cholesterol, which occur with increasing age, and the differences between the sexes, are in agreement with the findings of Russ, Eder and Barr (1) who applied Cohn fractionation and with those of Nikkilä (52) who used a paper electrophoresis technique.

The same development with increasing age that has been found for serum cholesterol and phospholipids appears to be valid for the β-lipoproteins. The large material from the Donner laboratory has been treated statistically and published by Tamplin and Tandy (2). It is based on observations from sera from non-fasting individuals in all groups below the age of 50 years, and comparisons must be made with reservation for the possible effects of this difference. Tamplin and Tandy demonstrated significant rises in all analyzed Sf classes (0—12, 12—20, 20—100, 100—400) in males, 23.6—43.4 years of age, but the Sf 0—12 and 12—20 classes showed larger increase in the interval 23.6—34.2 than in the interval 34.2—43.4. All classes remained constant after the age of 43.4. The largest recorded increases were found in another female material in the same paper in the age interval 33.4—44.1 years for all classes and in the interval 44.1—55.1 for the 20—100 and 100—400 classes. Up to the age of 50, the males as a whole showed higher values for the Sf classes below 20 than the females. The females then passed them and showed higher values for all classes with increasing age.

The younger groups in the present material cover the period when the males show higher concentration of the Sf classes over 20 than females in the material of Tamplin and Tandy. The older groups correspond to the post menopausal state in females compared with males of the same age.

The higher level of β-lipoprotein glyceride in the young males than in the young females is in good agreement with the distribution of Sf classes in the groups, as is the absence of a similar difference between the older groups.

The determination of the β-lipoprotein protein in the present investigation is a new feature in the work on lipoproteins and no comparable results are available for evaluation of the figures for the serum concentration of this component. Its increase with age is significant only in females, which would reflect the continuous increase in all lipoprotein fractions, which the Donner laboratory has found in females more than 50 years of age, while the males show stable levels of all fractions.

It is noteworthy that the β-lipoprotein lipid P does not show the same degree of increase with age in males as the β-lipoprotein cholesterol. The percentual increase in means between the age groups is 41 % for cholesterol, 21 % for lipid P and 11 % for the protein moiety. In females, the β-lipoprotein cholesterol increases by 85 %, the lipid P by 58 %, and the protein by 55 %. The lipid P and the protein follow one another more closely than the cholesterol and the protein.

The cholesterol/lipid P ratios in the younger groups were lower than those presented in a previous paper by this author (13) while there was no difference in the values for the older groups in the both studies. It is evident that the applied methods do not always yield comparable results.

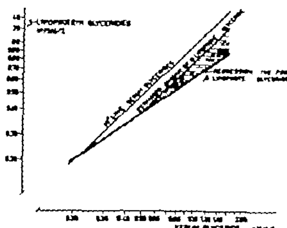


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The serum cholesterol and phospholipid values in the present normal material are within the range of values presented from the same region of Sweden (the west coast) by Svanborg and Svennerholm (21) and Malmcrona (44) but lower than those of Carlson (27) from Stockholm on the east coast. A multitude of factors, however have influence on the serum lipid values, such as dietary habits (29) seasonal variations (45), environmental factors, and "stress" (46, 47). All of these have not been taken into consideration in these materials. Moreover the increase in serum lipids is not continuous with age (48) and different age intervals of uneven rise have been found during investigations (49) The selection of narrow age limits for the normal groups may therefore lead to misleading results. Comparisons between different investigations seem justified *only if the investigations are carefully co*ordinated and carried out simultaneously

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with age and sex, it can be questioned if the judgement of the role of the lipoproteins in health and disease can be based only on quantitative estimations of the different lipoprotein classes, or on some of their lipid components. It should be useful to determine the relation between the lipid and protein moieties as well, and its possible significance in various clinical states.

Phospholipid distribution

Data on the distribution in the β-lipoprotein phospholipids have been published by Phillips (53) and by Nelson and Freeman (54). Both groups have separated the phospholipids on silicic acid columns after ultracentrifugal isolation of the lipoproteins. The ratio phosphatidyl P/sphingolipid P which can be calculated from their figures, after correction for the assumed molecular weight of phosphatidyl compounds (782) and sphingomyelin (721) lie within the same range as those of this investigation.

Phillips material is more fitted for comparison with the present figures. His fraction < 1.063 corresponds to the fraction isolated with the present method. He found a phospholipid composition corresponding to phosphatidyl sphingolipid ratio of 2.75 in the lipoproteins with a density < 1.063 in eight apparently healthy white adults and 3.07 for the fraction below 1.019.

The corresponding figures from Nelson and Freeman are 2.6 for Sf 0—20 and 4.46 for Sf 20—400. It should be noted that they selected sera from persons with elevated blood lipids in order to get sufficient amounts for the analyses.

The ratios of the present investigation are somewhat lower than Phillips except for the values in older males. There is no reason to assume a higher level of lipoprotein, < 1.019 in this group than in females of the same age. It seems probable that the present figures represent a sex difference in higher ages. It is difficult to understand the statement of Nelson and Freeman that the phospholipid distribution is constant regardless of the lipoprotein level in serum.

Correlation analysis

The correlation coefficients and the partial correlation coefficients indicate that cholesterol phospholipids, and protein form the frame-work of the lipoproteins, and that the glycerides are added to this in inconstant proportions.

Assume for moment that there exists a linear structural relationship of the type

$$C = aN + x, \quad P = bN + y$$

where C, P and N denote the quantities of cholesterol, lipid P and protein N while a and b are constants and x and y uncorrelated errors of measurement. Also assume that the glycerides, G are not correlated with C, P and N. The correlation coefficients will then satisfy the relations

$$r_{CP\cdot G} = r_{CP} \quad r_{CN\cdot G} = r_{CN} \quad r_{PN\cdot G} = r_{PN},$$
$$r_{CG} = r_{PG} = r_{NG} = 0.$$

The figures given in tables V—VI based on the evidence of the present limited material, lend some support to hypothesis of this kind.

The older females differ in this respect as their β-lipoprotein glycerides are as strongly correlated to the other components as are their β-lipoprotein cholesterol and lipid P. It should be observed that the present findings are valid for normal persons, and that the serum glyceride level varies within the narrow range of 0.20—1.58 mMol L.

A closer correlation between glycerides and lipid P might be expected, as the main part of the glycerides is found in the lipoproteins with a density of less than 1.006 which contain proportionately

Table VII Summary of results in literature of determinations of β-lipoprotein composition, including the protein moiety

Source	β-lipoprotein fraction	Molar ratio lipid/10 moles protein N			Cholesterol	Remarks
		Cholesterol	Lipid P	Glyceride	Lipid P	
Oncley 1954 (4)	1.026—1.045	2.70	1.13		2.39	
Hilliyard et al. 1955 (5)	< 1.063	3.41	1.34	1.6	2.54	Five males 27—50 years
Havel et al. 1955 (6)	< 1.019	3.10	1.89		1.64	Normal young adults
	1.019—1.063	3.91	1.38		2.83	Normal young adults
Bragdon et al. 1956 (7)	< 1.019	4.94	2.84	7.53	1.74	Eleven pooled sera
	1.019—1.063	3.25	1.25	0.46	2.60	Sixteen pooled sera
		3.70	1.30	0.26	2.85	Five normal males
Oncley et al. 1957 (8)	Sf 10—100	3.73	2.27		1.64	Pooled sera
	Sf 3—9	3.20	1.16		2.76	
	Sf 6—10	4.24	1.48		2.88	
Present work	Eluate from hydroxylapatite columns	2.60	1.09	0.43	2.39	15 normal males, 20—40 years
		3.33	1.14	0.39	2.86	11 normal males, 55—65 years

The following molecular weights have been used for the calculation of the molar ratios from the weight ratios: cholesterol 387, cholesteryl esters 669, phospholipid 775, triglyceride 850, nitrogen 14. Peptide has been converted to protein nitrogen by division by 6.25.

β-Lipoprotein lipid/protein relationships

Previous investigations with ultracentrifugal techniques on β-lipoprotein composition which include the protein moiety are listed in *table VII*. The values have been recalculated to molar ratios lipid/10 moles protein nitrogen. Only the values from Hilliyard et al. (5) are directly comparable with those of this investigation as these authors did not further separate the fractions of density < 1.063. Their values are close to those found in the older male group of the present investigation with the exception for the glyceride/protein ratio which is higher in their series, where the glycerides were determined according to the method total fatty acids — estimated phospholipid and cholesteryl ester fatty acids. There is also a striking resemblance between the values of Oncley (4) for the lipoprotein fraction 1.026—1.045 and the values for the younger male group in this study. Oncley et al. (8) however have demonstrated that the composition may vary also within very narrow Sf bands. Those given in the table are examples drawn from their paper and it can only be concluded that the present figures are comparable to those derived from ultracentrifugal studies.

The variation of the composition of the ultracentrifugally isolated lipoproteins makes it difficult to refer variations with age in the present results to changes in the distribution between the density classes. In view of the differences found

with age and sex, it can be questioned if the judgement of the role of the lipoproteins in health and disease can be based only on quantitative estimations of the different lipoprotein classes, or on some of their lipid components. It should be useful to determine the relation between the lipid and protein moieties as well, and its possible significance in various clinical states.

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6. The correlations between β-lipoprotein cholesterol, lipid P and protein are significant in all groups, and a close *structural* relationship between these components appears to exist. The glycerides do not show the same degree of correlation.

Partial correlation analysis confirms these findings.

Acknowledgement

Thanks are due to Mrs Franca Widlund, Miss Anita Jacobsson, Miss Barbro Carlander and Mr Peter Bedding for skilful technical assistance, and to Mrs Marianne Sylvén for untiring secretarial aid.

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Partial correlation analysis confirms these findings.

Acknowledgement

Thanks are due to Mrs Franca Widlund, M[illegible] Anita Jacobsson, Miss Barbro Carlander a[illegible] Mr Peter Bedding for skilful technical assistan[illegible] and to Mrs Marianne Sylvén for untiring sec[illegible]tarial aid.

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Serum β-Lipoprotein Lipids and Protein during Combined Administration of Dioxydiethylstilbestrol and Methyl Testosterone

By

K. Cramér

Previous investigations (1—4) ha e shown that a combination of a synthetic estrogen and methyl testosterone causes a sharp rise in the serum β-lipoprotein cholesterol or in the concentration of low density lipoproteins. The author has demonstrated that the rise is more marked in the lipoprotein phospholipids than in the β-lipoprotein cholesterol (5) How ever the pplied technique (6) did not allow estimations of changes in the glyceride or protein moieties of the lipoproteins This investigation as carried out to define these changes.

Clinical material

F men, 69—82 years of age were selected from those treated with dioxydiethylstilbestrol (DDS) for prostatic disease at Department III of Vasa Sjukhus in Göteborg. Three had benign enlargement of the prostate while tw suffered from prostatic carcinoma. All fi had an erythrocyte sedimentation rate below 20 mm/hour and hemoglobin values exceeding 12.0 g 100 ml. The serum acid phosphatase was below 3.0 Buch units. This is the upper limit of normal w th the applied method.

Methods

The patients had been on treatment with DDS 5 mg 3 daily for least 6 weeks before the combined treatment w th DDS and methyl testosterone (MTEST). MTEST was given sublingually in dose of 10 mg 3 i two patients and 5 mg 2 in three patients. All serum samples were drawn in the morning They were examined the day when MTEST was first given, and on the 7th, 14th, and 21st day of combined treatmen with DDS and MTEST

The patients were kept on the ordinary hospital diet, the fat content being mainly saturated fat of animal origin. The calories derived from fa eraged 40 of the total daily supply The diet remained unchanged during the study

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Supported by grants from the Swedish National Association for the Study of Heart and Lung Diseases and the Swedish Margarine Manufacturers Association.

Results

The percentage changes in the serum lipids are shown in fig 1 None of the serum lipids changed significantly during the study

The development in the β-lipoprotein components (fig 2) showed a significant rise in total cholesterol ($P < 0.05$) lipid P ($P < 0.02$) and protein nitrogen ($P < 0.01$) after one week. During the second week the elevation continued but the dispersion in cholesterol values was considerable whereas the lipid P was significantly increased ($P < 0.001$) in all patients. Cholesterol, on the other hand was not significantly increased, and a wide dispersion of the val ues was found. Protein nitrogen reached the highest level of all components, 146 over the initial level ($P < 0.01$) None of these components were significantly more elevated than the others. During the third week, a return towards the initial value was observed, and the resulting increases t this point were insignificant except for lipid P ($P < 0.05$) The glyceride glycerol level remained constant during the first two weeks and declined during the third week. The decrease during the third week wa insignificant owing to the dispersion of the values.

Tests with double diffusion-in-gel pre cipitation according to Ouchterlony as modified by Wadsworth (16) revealed no protein contaminations in th β-lipo-protein preparations during the study

The mean levels of the serum lipids and the β-lipoprotein components during treatment with DDS alone and during the ourse of the combined treatment with DDS and MTEST are given in table I—II. Molar relations within the β-lipoproteins are gi en in table III In all tables the values for 11 normal men, 55—65 years of age (13) are given for comparison.

It was found that the increase of the protein nitrogen during MTEST was significant ($P < 0.05$) while the other components did not significantly differ from their levels during treatment with DDS. The glyceride/protein ratio was decreased ($P < 0.01$) but except for this the composition of the β-lipoproteins was unchanged

The mean ratios cholesterol/protein and cholesterol lipid P in the β-lipo-proteins were considerably lo er in this material than n the normal males 55—63 years of age D rect comparison, however was not possible owing to the age difference between the groups.

Discussion

No side effects were observed during the administration of MTEST There were no relapses from the disease of the prostate and th acid and alkaline phosphatase level in serum remained unchanged.

It was difficult to control night-eating n these patients, and t is possible that some of them ate chocolate or candy during the night before the specimen was drawn, which would primarily in-fluence the serum glyceride values (13)

46 of the serum phospholipids were transported with the β-l poproteins dur ing the treatment with DDS This pro-portion increased to 65 % when MTEST was added to DDS although the serum phospholipid level remained unchanged. This is quite in accordance with the findings of Russ, Eder and Barr (1)

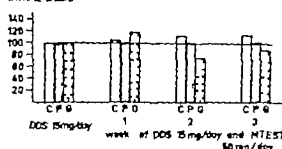


Fig 1 Percentage changes of serum lipids during combined administration of DDS and MTEST
C = total cholesterol.
P = phosphol pids.
C = glycerides.
No significant changes were observed.



Fig 2. Percentage changes of β-lipoprotein components during combined administration of DDS and MTEST
C = total cholesterol
P = phospholipids.
G = glycerides.
N = protein nitrogen.
The differences against the initial level were tested by the t test and found significances indicated with P values above the columns. No P values are indicated where no significant difference was found.

Table I Mean serum lipid levels in five men during treatment with DDS and during combined administration of DDS and MTEST during three weeks. Values for 11 normal men, 55—65 years old, are given for comparison

Serum Mean level in 5 men, 69—82 years old	Total cholesterol mg/100 ml	Phospholipids mg/100 ml	Glyceride glycerol mMol/L
During treatment with DDS 15 mg/day	200±11	257±15	1.62 1.27—2.07
During combined treatment with DDS, 15 mg day and MTEST 50 mg day 3 observations in each person	217±10	24 ±8	1.27 1.07—1.50
Mean level for 11 normal men, 55—65 years old (15)	214±9	236±10	0.86±0.11

The values for serum glyceride glycerol were converted into logarithms before statistical calculation. The reconverted values of the mean for log glyceride ± the standard error of this mean are given in the table

No significant differences were found between the values before and after addition of MTEST

Analyses were mad for serum total cholesterol, phospholipid, and glyceride glycerol as well as for β-lipoprotein cholesterol, lipid P glyceride glycerol, and protein nitrogen. The β-lipoprotein phospholipids were also examined to determin the distribution between phosphatidyl compounds and sphingolipids.

Total cholesterol was d termined according to Theorell, as revised by Cramér and Isaksson (7). The values were reduced by 4 6 % to make them comparable (7 8) with values determined according to Sperry and W bl (9)

Lipid phosphorus was determined according to S anborg and Svennerholm (10), glyceride glycerol according to Carlson and Wadstrom (11) simplified by Carlson (12) Phospholipid hydrolysis and partition and protein nitrogen determination by a micro-Kjeldahl procedure ha been described in a previous paper (13)

The β-lipoproteins were isolated by chromatography on hydroxylapatite columns (14 15 13)

The standard error of all methods, as well as an outline of the presentations of the results, are gi en in a previous paper (12) Th statistical calculations have been performed as described by Kemp (16

Oliver and Boyd (2) and Furman, Howard, Norcia and Keaty (3) that the α-lipoproteins are increased during estrogen treatment and decreased when synthetic androgens are added

The range of absolute values makes a statistical evaluation less reliable. The only difference found between the levels during DDS therapy and combined DDS + MTEST administration was in the rise in the β-lipoprotein protein moiety which was significantly increased at the 5 per cent level. The percentage changes from week to week of MTEST administration, on the other hand showed the largest increases in the phospholipid and protein moieties. It is known from the studies of Kochakian (18) that MTEST has a protein anabolic effect. It can at present not be stated if its lipid-elevating effect is secondary to the increase in lipoprotein protein or independent of this.

The previous finding of a larger increase in β-lipoprotein phospholipids than in the cholesterol component (5) was confirmed, although there was no significant depression of the β-lipoprotein cholesterol/lipid P ratio in the present material when calculated on the mean of the individual ratios.

The phospholipid fractions did not change in relation to one another

The decrease in β-lipoprotein glyceride would indicate relative increase of the lipoproteins of the 1.019—1.063 density class. However this decrease is not concomitant with an elevated cholesterol/lipid P ratio in the β-lipoproteins such as could be expected when a relative increase in the lipoproteins of the above values occurs. This confirms previous assumptions (5) of a change in lipoprotein composition during administration of MTEST

Summary

1 Administration of dioxydiethylstilbestrol (DDS) to patients with prostatic disease evokes lipid changes similar to those in survivors from myocardial infarction reported by other authors.

2 Addition of methyl testosterone (MTEST) to the treatment with DDS gives an increase in all β-lipoprotein components except the glycerides.

3 The increase is most marked in the protein and the phospholipid components of the β-lipoprotein and during the second week of the administration of MTEST

4 The glyceride component of the β-lipoproteins is depressed by MTEST and the glyceride/protein ratio significantly lower than during treatment with DDS

5 It can not be excluded that the lipid changes are secondary to the increase in β-lipoprotein protein.

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Table II Serum concentration of β-lipoprotein components in five men during treatment with DDS and during combined administration of DDS and MTEST during three weeks. Values for 11 normal men, 55—65 years old, are given for comparison

Mean level in 5 men, 69—82 years old	Serum concentration of β-lipoprotein components					
	Cholesterol		Phospholipids		Glyceride glycerol	Protein nitrogen
	mg/100 ml	mMol/L	mg/100 ml	mMol/L	mMol/L	mMol/L
During treatment with DDS, 15 mg/day	127 120 — 134	3.28 3.11 — 3.47	115 103 — 129	1.49 1.33 — 1.67	1.14 1.08 — 1.19	12.63 11.61—13.7
During combined treatment with DDS 15 mg/day and MTEST 50 mg/day 3 observations in each person	153 144 — 163	3.96 3.73 — 4.21	145 133 — 157	1.87 1.71 — 2.03	0.88 0.79 — 0.97	16.48 15.45—17.58 < 0.05
Mean level for 11 normal men, 55—65 years old (13)	173 ± 10	4.48 ± 0.26	123 ± 7	1.61 ± 0.09	0.56 0.49 — 0.63	13.89 ± 0.83

The distribution for the β-lipoprotein components was skew and the values in the DDS and DDS + MTEST treated groups converted into logarithms before statistical calculation. The reconverted values for the mean ± the standard error of the mean are gi en in the table. All other values are given as the mean ± the standard error of the mean.

The p-value refers to tests between the groups before and after addition of MTEST

No other significant differences were found.

Table III β-Lipoprotein molar ratios in five men during treatment with DDS and during combined administration of DDS and MTEST during three weeks. Values for 11 normal men, 55—65 years old are given for comparison

Mean level in 5 men, 69—82 years old	β-Lipoprotein molar ratios				
	Ratio lipid/10 moles protein N			Cholesterol / Lipid P	Phosphatidyl P / Sphingolipid P (4 cases)
	Cholesterol	Lipid P	Glyceride		
During treatment with DDS, 15 mg/day	2.66 ± 0.28	1.19 ± 0.07	0.90 0.76 — 1.06	2._7 ± 0.23	2.4? ± 0.17
During combined treatment with DDS 15 mg/day and MTEST 50 mg/day 3 observations in each person	2.43 ± 0.08	1.15 ± 0.06	0.53 0.49 — 0.58 < 0.01	2.19 ± 0.12	2.64 ± 0.20
Mean level for 11 normal men, 55—65 years old (13)	3.33 ± 0.07	1.14 ± 0.05	0.39 0.35 — 0.45	2.80 ± 0.07	2.86 ± 0.17

The values are given ± the standard error of th mean.

The values for th glyceride/protein ratios were con erted int logarithms before statistical calculation. The reconverted values of the mean for log glyceride protein ± the standard error of this mean are given in the table.

The p-value refers to tests between the groups befor and after addition of MTEST

N other significant differences were found.

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Serum β-Lipoprotein Lipids and Protein during Administration of Triparanol

By

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Steinberg, Avigan, and Feigelson (1) have demonstrated that triparanol[1] decreases the true serum cholesterol level by about 30 % of the initial value, but that a simultaneous accumulation of the cholesterol precursor desmosterol in serum limits the net decrease in total serum sterol to 15 %. A great number of publications, recently reviewed by Furman and Robinson (2), have shown that the formation of desmosterol leads to lower values for total serum cholesterol in determinations using the common Liebermann-Burchard reaction, as desmosterol has not the same molar extinction as true cholesterol.

No data have so far been published about the effect of triparanol on lipoproteins in human serum.

This investigation has been carried out to determine the changes within β-lipoproteins when triparanol is administered and when the cholesterol level in serum is depressed.

[1] 1-(p-[β-diethylaminoethoxy]phenyl)-1-(p-tolyl)-2-(p-chlorophenyl)ethanol, kindly supplied by Merrell National Ltd through the courtesy of AB Hassle, Göteborg, Sweden.

Aided by grants from the Swedish National Association for the Study of Heart and Lung Diseases.

Submitted for publication October 2, 1961

Clinical material

Five women, 49—64 years of age, who had serum cholesterol levels exceeding 280 mg/100 ml, who had been controlled with cholesterol determinations for at least two years before triparanol was tried. Three of them had typical tendinous xanthomata. All five had been subjected to unsuccessful attempts to depress serum cholesterol by different dietary measures such as a fat-restricted diet or diets based on polyunsaturated fatty acids. They were accustomed to using polyunsaturated fat in their cooking and were instructed not to change anything in their diet during the course of the triparanol treatment. Mean values from 11 normal women of the same age group (9) are given for comparison.

Methods

Triparanol was taken in capsules of 250 mg. One capsule was taken before breakfast. Three of the patients complained of constipation in connection with the treatment, but no other side effects were observed. Blood was drawn in the fasting state on two separate days before treatment and on two separate days after 31—40 days of triparanol treatment.

Total cholesterol was determined according to Theorell, as revised by Cramér and Isaksson (3). The values were reduced by 4.6 % to make them comparable (3, 4) with values determined according to Sperry and Webb (5

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35—65 years of age

β-lipoprotein					Protein N mMol/L
Cholesterol		Phospholipids		Glyceride glycerol mMol L	
mg 100 ml	mMol L	mg/100 ml	mMol L		
261±19	6.74±0.50	190±14	2.45±0.18	0.62 0.56—0 69	20 86±1.51
192±17	4 96±0.44	177±13	2.28±0.17	0.86 0 66—1 15	21 13±1 79
<0 03					
187±11	4.83±0.29	139±11	1.79±0.11	0.67 0 59—0.77	16 13±0.86

The values for β-lipoprotein cholesterol and phospholipid are given in mg 100 ml as well as in mMol L.
The P values refer to t-tests made between the values before and after triparanol.

Results

One patient did not respond at all to the treatment, but showed a slight rise in serum cholesterol and β-lipoprotein level. She was 64 years of age and suffered from hereditary hypercholesterolemic xanthomatosis. As the purpose of the investigation was to determine the changes within the β-lipoproteins during actual decrease of serum cholesterol by triparanol, the values observed in this patient were omitted in the calculations listed below.

The results from the remaining four patients are given in tables I and II and a typical case is shown in fig 1.

The serum cholesterol showed a decrease of 75 mg/100 ml as the mean value ($P < 0.02$) while the change in serum phospholipid was less obvious. The serum glycerides increased in two patients but remained constant in two. β-Lipoprotein "cholesterol" was decreased by 69 mg 100 ml, the primary change in serum "cholesterol" was found in this fraction. There was no change in the phospholipid or protein moieties of the β-lipoproteins. A slight elevation of the glyceride moiety was observed. This was parallel to the observed serum glyceride increases in two patients.

The proportions between the components of the β-lipoproteins changed accordingly. The cholesterol /protein ratio was significantly decreased ($P < 0.01$) while the lipid P/protein and glyceride protein ratios remained constant.

The β-lipoprotein cholesterol lipid P ratio was decreased ($P < 0.01$). No changes in the composition of the β-lipoprotein phospholipids were observed.

Discussion

The determination of "cholesterol" during treatment with triparanol did not give values for true cholesterol but for the sum of cholesterol and desmosterol. Desmosterol did not give the same colour intensity in the Liebermann-Burchard reaction as cholesterol (1) but its exact chromogenity in different variations of this method was not determined. It was impossible to obtain desmosterol for standardization purposes, and thus desmosterol was not determined in this study. The determinations of cholesterol during the treatment with triparanol were compared with determi-

Table I Serum and β-lipoprotein lipids before and after treatment with triparanol and in normal women

Mean level in 4 women, 2 observations on each	Serum		
	Cholesterol mg/100 ml	Phospholipids mg/100 ml	Glyceride glycerol mMol L
Before triparanol	324±17	318±12	0.78 0.67—0.90
After triparanol 250 mg × 1 31—40 days	249±19 < 0.02	290±11	1.13 1.00—1.62
Mean for 11 normal women, 55—65 years	261±9	274±6	0.96 0.87—1.07

The values are given ± the standard error of the mean.

The values for glycerid glycerol were converted into logarithms before statistical calculation. The reconverted values of the mean for log glycerides ± the standard error of this mean are given in the table.

Table II Composition of β-lipoproteins before and after triparanol and in normal women, 55—65 years of age

Mean value in 4 women, 2 observations on each	β Lipoprotein molar ratios				
	Lipid 10 mol protein N			Cholesterol / Lipid P	Phosphatidyl P / Sphingolipid P
	Cholesterol	Lipid P	Glyceride glycerol		
Before triparanol	3.25±0.16	1.18±0.03	0.30 0.27—0.33	2.76±0.09	2.16±0.12
After triparanol 250 mg × 1 31—40 days	2.38±0.20 < 0.01	1.09±0.04	0.42 0.34—0.51	2.18±0.14 < 0.01	2.19±0.09
Mean for 11 normal women, 55—65 years	3.13±0.03	1.11±0.02	0.41 0.38—0.45	2.71±0.05	2.36±0.10

The values are given ± the standard error of the mean.

The values for the glyceride protein ratios were converted into logarithms before statistical calculation. The reconverted values of the mean for log glyceride protein ± the standard error of this mean are given in the table.

The P values refer to t-tests made between the values before and after triparanol.

Lipid phosphorus was determined according to Svanborg and Svennerholm (6), glyceride glycerol according to Carlson and Wadström (7) simplified by Carlson (8). Phospholipid hydrolysis and partition and protein nitrogen determination by a microKjeldahl procedure have been described in a previous paper (9).

The β-lipoproteins were isolated by chromatography on hydroxylapatite columns (10, 11, 9).

The standard error of all methods, as well as an outline of the presentations of the results, are given in a previous paper (9). The statistical calculations have been performed as described by Kemp (12).

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Addendum

After the completion of this work, a description of a method for the determination of desmosterol, worked out at the Merrell Co., became available. It is a modification of the method of Abell et al (15) where the extinction is read at 620 mμ and 400 mμ respectively and the amount of desmosterol calculated after a given formula. Normal sera should give a desmosterol value of 0. A desmosterol standard has still not been available.

Determinations with this method, carried out in quadruplicates, were made on β-lipoprotein preparations from three subjects after a 30 days period of 250 mg triparanol daily. The results are given in table III compared with the values from the same patients before treatment. The cholesterol determinations before treatment were determined according to Theorell, as revised by Cramér and Isaksson (3). Sera and β-lipoprotein preparations from untreated persons gave a desmosterol value of 0.

The sum of cholesterol and desmosterol gave about the same value as that for cholesterol before treatment. Between 13 and 38 % of the sum was represented by desmosterol. It was thus confirmed that desmosterol enters into the β-lipoprotein molecules and replaces cholesterol. The level of the other β-lipoprotein components remained unchanged or were elevated.

Table III. Molar concentrations of β-lipoprotein components before and after treatment with 250 mg triparanol/day. Determinations of cholesterol and desmosterol were only made after the treatment

Subject	Before triparanol*				After triparanol 250 mg/day 30 days*						Desmosterol as per cent of cholesterol + desmosterol
	Cholesterol	Phospholipids	Glycerides	Protein nitrogen	Cholesterol	Desmosterol	Cholesterol + desmosterol	Phospholipids	Glycerides	Protein nitrogen	
HS	7.17	2.92	0.78	23.62	4.99	1.60	6.59	2.77	2.90	25.63	24
EN	7.93	2.80	0.70	25.33	6.83	1.06	7.89	2.64	1.70	26.63	13
MP	7.30	2.34	0.33	18.66	5.02	3.03	8.05	3.11	0.50	23.20	38

* All values are expressed as mMol/L, mean of two determinations, one sample from each subject.

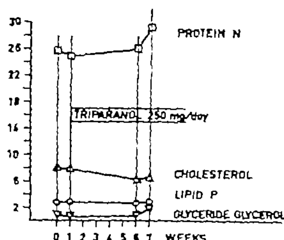


Fig 1 Effect of triparanol on β-lipoprotein components. All concentrations are given in mMol/L.

nations according to Sperry and Webb (5). No other difference in values than described earlier (3, 4) was found.

The β-lipoprotein cholesterol was 81 % of the total serum cholesterol before triparanol. 92 % of the decrease of cholesterol during triparanol was found in this fraction.

It is difficult to evaluate the significance of the changes found in the β-lipoprotein molecules. It is known (1 unpublished results) that desmosterol can be incorporated in lipoprotein molecules as can cholesterol. The other β-lipoprotein components have remained at a constant level in this study. This would either mean that the β-lipoproteins had a proportionately low cholesterol content or that they carried desmosterol instead of cholesterol. It is known that desmosterol is formed during administration of triparanol, and that it must be kept in solution in some lipoprotein complex. It seems likely that it replaced cholesterol in the β-lipoproteins. The mechanism that regulates the level of the other β-lipoprotein components in serum was however quite unaffected.

The composition of the β-lipoproteins varies with age and sex (9) and can be influenced by experimental or therapeutic measures (13). There is considerable evidence that the β-lipoproteins should be regarded as the noxious agent in atherosclerosis (14), but there is no proof that this is caused by their cholesterol component.

It seems doubtful that the changes observed after triparanol should be beneficial to the patient with regard to the prevention of atherosclerosis.

Summary

1. The effect of triparanol on serum lipids and on the components of the serum β-lipoproteins has been studied in five women who had serum cholesterol levels exceeding 280 mg/100 ml.

2. Triparanol had no effect in one case. In the four remaining cases, a mean decrease in chromogenity in the Liebermann-Burchard reaction equivalent to 75 mg/100 ml of cholesterol was observed.

3. This reduction occurred primarily within the β-lipoproteins. The phospholipid and protein moieties did not change their levels.

4. A hyperglyceridemic condition was induced in two patients, but the mean level of serum or β-lipoprotein glycerides was not significantly changed.

5. The ratio cholesterol/protein in the β-lipoproteins was significantly decreased ($P < 0.01$) as was the cholesterol/phospholipid ratio. The lipid P/protein and glyceride/protein ratios were not changed.

6. The results indicate that triparanol causes a partial replacement of cholesterol with desmosterol, but that the actual level of β-lipoprotein is not changed.

7. It has not been established that such a change would be a preventive measure against atherosclerosis.

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Changes in Serum and Lipoprotein Fatty Acids during Administration of Ethyl Arachidonate

By

K. Cramér and P. Björntorp

The distribution of the polyunsaturated fatty acids (PUFA) in serum has been determined in several reports (for review see Björntorp (1) and Schrade et al. (2)) while only a limited number of publications deal with their distribution within the lipoproteins of serum (3—6). No detailed report on the effect of administration of polyunsaturated fatty acids on this distribution has been published as yet. The present study was performed in order to determine the effect of arachidonic acid[1] which normally is synthesized in the animal or human from linoleic acid (7).

Clinical material

This study has been performed on two male subjects.

E. L., born 1912, was a heavy-duty labourer until 1957 when he got typical angina pectoris. An ECG after exercise revealed a coronary insufficiency. He never had ECG changes at rest. Since then he has been employed as a watchman. Serum total cholesterol was determined in 1959 and was 371 mg/100 ml. It was reduced to a level of 320 mg/100 ml by dietary fat restriction, and to 280 mg/100 ml by addition of linoleic acid as oleomargarine (Margo, Margarinbolaget, Stockholm). The administration of linoleic acid was interrupted 2 months before he was admitted into the hospital for the study. He suffered an attack of nephrolithiasis 1 month before admission, but had no symptoms of this during the study.

L. H., born 1918, suffered a viral hepatitis in 1947 and a duodenal ulcer in 1952. An acute posterior myocardial infarction was diagnosed in 1954 and he has had moderate anginal pain on effort since then, but is regularly at work as a headwaiter. His serum lipids have not been controlled regularly but a serum cholesterol value of 335 mg/100 ml was registered three weeks after the infarction 1954. H. has not kept any special dietary regimen to lower the serum lipids.

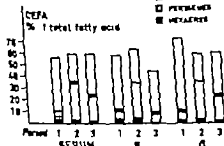
[1] Δ 5,8,11,14-eicosatetraenoic acid as the ethyl ester by F. Hoffmann-La Roche & Co., Basle, Switzerland. The preparations held not more than 10 per cent of other fatty acids.

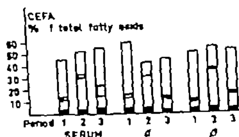
Submitted for publication October 7, 1961

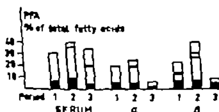
Supported by grants from the Swedish National Society for the Study of Heart and Lung Diseases and from the Swedish Margarine Manufacturers' Association, which also supplied the Margo oleomargarine.

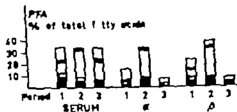
Table II. Total amounts of serum fatty acids before and after administration of ethyl arachidonate

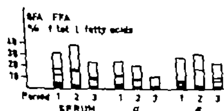
Serum fatty acids in mg/100 ml at the end of period	E. L.				L. W.			
	CEFA	PFA	GFA+FFA	Total	CEFA	PFA	GFA+FFA	Total
1	180	102	65	347	202	134	116	452
2	105	89	62	256	134	114	82	330
3	96	66	58	220	116	95	107	318











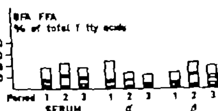


Fig. 1 Fig. 2.

Percentage distribution of PUFA in E. L. (left) and L. W. (right) before administration of ethyl arachidonate.

Period 1 = basal diet + 80 g butter fat.
Period 2 = basal diet + 60 g butter fat + 20 g ethyl arachidonate.
Period 3 = basal diet + 75 g oleomargarine + 5 g ethyl arachidonate.
α = α-lipoprotein lipids.
β = β-lipoprotein lipids.

Table I. Serum lipids before and after administration of arachidonate

Period	E. L.		L. W.	
	Total serum chol. mg/100 ml	Serum glyc. mMol/L	Total serum chol mg/100 ml	Serum glyc. mMol/L
1 n = 5	294 ± 1	0.95 ± 0.09	348 ± 19	1.53 ± 0.20
2	224	0.60	300	0.98
3 n = 6	242 ± 4 < 0.01	0.88 ± 0.08	348 ± 9	1.09 ± 0.08

last value of the period owing to its briefness.
n = number of observations.
The P value refers to t-tests between the values from periods 1 and 3. No other significant differences were found.

Methods

Both subjects who were very cooperative were admitted into the hospital during the first two periods of the study but were not kept in bed. They were given a diet containing an average of 2,200 calories a day with 40 % of the calories derived from fat, 20 from protein, and 40 % from carbohydrate.

The diet contained only minor amounts of vegetable fat, as the consumed fat originated from meat or from butter. The daily amount of butter was 80 g. This regimen was continued for 18 days and will be referred to as *period 1*.

After 18 days, 70 g of ethyl arachidonate was substituted for the equal amount of butter but no other changes were made in the diet. This regimen was kept for 10 days, after which the supply of arachidonic acid was exhausted. This period is referred to as *period 2*.

Both subjects were then controlled as out patients. Basically they kept the same diets as during periods 1 and 2 and had been especially instructed not to change the proportions between fat, protein, and carbohydrate. Fat was partially obtained from oleomargarine "Margo" containing 45 per cent of linoleic acid, and partially from meat. The results from this period, which lasted 37 days, will not be dealt with in this paper.

Then, arachidonic acid was again available and *period 3* started with a dose of 10 g of ethyl arachidonate daily for 14 days, followed by a period with 5 g daily for 37 days. The remaining fat was from meat and from oleomargarine ("Milda" Margarinbolaget, Stockholm) with a low content of PUFA. The total calorie content and the proportions fat : protein : carbohydrate calories were the same as during periods 1 and 2. The average daily supply of linoleic acid was calculated to 5 g and of linolenic acid to 1 g.

All blood samples were drawn in the fasting state. They were examined for serum total cholesterol and glycerides. The last specimen in each period was fractionated into α-lipoproteins and β-lipoproteins, and liped extracts from these fractions and from serum were subjected to silicic acid chromatography to isolate the cholesteryl esters, phospholipids, and glycerides + free fatty acids. The polyunsaturated fatty acids (PUFA) in these fractions as well as the total amount of fatty acids in each fraction were then determined. They will be abbreviated as CEFA = cholesteryl ester fatty acids, PFA = phospholipid fatty acids, and GFA + FFA = glyceride fatty acids + free fatty acids.

β-Lipoprotein isolation was performed by chromatography on hydroxylapatite according to Hjertén (8) as further developed by Cramér and Brattsten (9) and Cramér (10).

The preceding 0.25-M potassium phosphate buffer from the hydroxylapatite columns was first dialyzed over night against running tap water and then concentrated in the dialysis bag in a stream of air of room temperature to a volume of about 20 ml. The lipids were extracted from the concentrated solution with chloroform-methanol 1 : 1 (v/v) and diluted to 250 ml. Studies of the chromatography with hydroxylapatite have shown that this fraction is not contaminated with β-lipoproteins and that it represents the serum lipids except the β-lipoprotein lipids and probably also except the chylomicron fraction in serum (10). It has been denoted as α-lipoprotein lipids.

The lipids were separated by means of *silicic acid chromatography* according to Hirsch and Ahrens (11) and *determination of PUFA by alkali isomerization* made according to Holman and Hayes (12).

Comparison between periods 1 and 2

The CEFA showed a three- to tenfold increase of the percentage of tetraenes after period 2, simultaneous with a reduction of the dienes to generally less than one half of their percentage value of the end of period 1. These changes were seen in both patients and in all examined lipoprotein fractions. The PFA changed in the same manner as the CEFA, but the changes were generally even more obvious.

The GFA + FFA differed from the other fractions. There was an increase in percentage tetraenes in serum, but no reduction in dienes. The increase in tetraenes took place within the β-lipoproteins, while the α-lipoprotein GFA + FFA showed no changes of their tetraene percentage.

Results after period 3

The percentage PUFA in the PFA of the lipoprotein fractions decreased in both patients to less than 10 during period 3, while it was 34 in the serum. There were some elevations of the tetraenes as compared with the end of period 1, more marked in E. L. than in L. W., as well as a tendency toward lower diene percentages.

Discussion

Björntorp (1) demonstrated that the alkali isomerization technique compares well with gas-liquid chromatography and that the values for serum obtained by him were within the same range as those from other laboratories.

It is more difficult to find figures for comparison concerning the PUFA of the lipoprotein fractions. No large material has been published. Green, Oncley and Karnovsky (5) and Lindgren, Nichols and Wills (6) have given values similar to those of the present study, period 1, with the exception for the dienes in the PFA fractions, which are higher in their studies, as well as the tetraenes in the α-lipoprotein PFA. The diet of the persons studied by Green et al. is not specified, while the non-fasting subjects studied by Lindgren et al. kept a house diet. Nelson and Freeman (4) fractionated the phospholipids from the Sf classes 0—20 and 20—400 into lecithin and sphingomyelin and determined the fatty acids of these fractions. They selected 2 patients with high serum lipids to be certain to get sufficient amounts for analyses. The results showed greater individual differences than those between the two subjects of the present study.

The effect of highly unsaturated fat on serum lipids has been demonstrated by Ahrens et al. (19), Kingsbury et al. (20) and Kinsell et al. (21). Our study confirms the effect of arachidonic acid, as demonstrated by Kingsbury et al. A dose of 5 g daily, however, was not sufficient to decrease the total cholesterol level in serum in one of the subjects, L. W., who had shown a decrease on a dose of 20 g daily. The other subject, E. L., reacted well also on the lower dosage.

The source of the CEFA PUFA is generally dietary (22). Thus, when corn oil is given, the CEFA contain a high concentration of linoleic acid, and when menhaden oil is given the CEFA also resemble the dietary fat in composition (19). Under ordinary dietary conditions linoleic acid is the main dietary PUFA. The dietary supply of linoleic acid was very low during period 1 of the present study and remained so during period 2, when arachidonic acid was given. The resulting changes in the CEFA are in accordance with the findings of Ahrens

Table III. Percentage amount of dienes (II) and tetraenes (IV) in serum and in serum lipoproteins before and after administration of ethyl arachidonate

Fraction		Serum			α-lipoproteins			β-lipoproteins			
Period		1	2	3	1	2	3	1	2	3	
CEFA	II	55	23	34	45	28	34	60	23	35	E. L.
	IV	3	31	21	6	30	7	6	32	20	
PFA	II	24	4	14	16	5	4	9	9	4	
	IV	2	27	15	1	14	1	6	20	2	
GFA+FFA	II	14	21	13	12	11	8	20	13	12	
	IV	7	12	6	7	6	1	2	14	5	
CEFA	II	31	19	31	43	11	31	39	18	35	L. W.
	IV	8	27	10	9	27	10	6	31	12	
PFA	II	11	1	9	9	4	4	8	4	4	
	IV	9	23	15	2	18	2	6	24	2	
GFA+FFA	II	10	10	8	16	6	8	8	9	8	
	IV	2	8	4	4	5	1	2	8	4	

Total cholesterol was determined according to Theorell as revised by Cramér and Isaksson (13). The values were reduced by 4.6 % to make them comparable (13, 14) with values determined according to Sperry and Webb (15).

Lipid phosphorus was determined according to Svanborg and Svennerholm (16) and *glyceride glycerol* according to Carlson and Wadström (17) as simplified by Carlson (18).

Results

The diet was generally well tolerated by both subjects, although the abundance of butter during periods 1 and 2 caused some discomfort. L. W. who was overweight at the beginning of the study lost 4 kg during periods 1 and 2 but then remained at constant weight until the end of period 3. E. L. kept his weight constant during the whole study. The introduction of ethyl arachidonate caused some gastrointestinal disturbance in both subjects with frequent stools and eructations. The discomfort was never severe and was overcome after about a week.

The serum cholesterol and glycerides changed as is shown in table I. Both subjects showed a decrease in these lipids during period 2. During period 3 the serum cholesterol values for E. L. were significantly lower than during period 1 while the glycerides remained constant. During period 3 L. W. showed values which, basically, were almost similar to those shown during period 1.

The serum total fatty acids in the different lipid classes are given in table II. The *sum of the* fatty acids of the fractions dropped from the end of period 1 to the end of period 2 but remained more or less constant during period 3. The GFA + FFA in serum did not show a change, which was parallel to the other fractions.

The percentages of PUFA in the fatty acids of the lipid fractions in serum and in the α- and β-lipoproteins are seen in fig. 1 and fig. 2 and the percentages of dienes and tetraenes are given in table III.

Comparison between periods 1 and 2

The CEFA showed a three- to tenfold increase of the percentage of tetraenes after period 2, simultaneous with a reduction of the dienes to generally less than one half of their percentage value of the end of period 1. These changes were seen in both patients and in all examined lipoprotein fractions. The PFA changed in the same manner as the CEFA but the changes were generally even more obvious.

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The source of the CEFA PUFA is generally dietary (22). Thus, when corn oil is given, the CEFA contain a high concentration of linoleic acid, and when menhaden oil is given the CEFA also resemble the dietary fat in composition (19). Under ordinary dietary conditions linoleic acid is the main dietary PUFA. The dietary supply of linoleic acid was very low during period 1 of the present study and remained so during period 2 when arachidonic acid was given. The resulting changes in the CEFA are in accordance with the findings of Ahrens

et al (19) that the CEFA mirror the dietary fatty acid composition closely

The most pronounced changes, however occurred in the PFA. An ordinary diet is concomitant with a higher percentage of arachidonic acid in the PFA than that found after period 1 of the present study (1 5 6) The percentage of arachidonic acid has also been found higher in the PFA than in the CEFA This, and the striking changes when arachidonic acid is given suggest a specific metabolic relationship between arachidonic acid and the serum phospholipids.

It should be noted that Mead and Fillerup (23) in contradiction to Ahrens et al. (19) found that linoleic acid was rapidly incorporated in the serum phospholipids while stearate and oleate were found in the glycerides. If this characteristic of linoleic acid is shared by other PUFA *e g* arachidonic acid this might explain the finding of a high concentration of arachidonic acid in the PFA.

The GFA in the α-lipoproteins showed the least pronounced changes when arachidonic acid was given. This is a small lipid fraction and its relation to the dietary fatty acids seems to be of minor importance. No striking differences were otherwise found between the lipoprotein fractions when the response to the dietary change was followed

Mead (7) has also established the metabolic sequence from linoleic acid to arachidonic acid If this is an important function of linoleic acid a high dietary supply of arachidonic acid might make a mobilisation of linoleic acid from the fat depots unnecessary and thus reduce the level of this acid in the metabolically most active lipid fractions.

On the basis of experiments with animals who had a deficiency of PUFA and on the basis of determinations of PUFA in serum from normal young adults, Holman, Hayes, Malmros and Wigand (24) and Holman (25) formulated the theory that cholesterol and PUFA were needed for the transport of saturated fatty acids, and that a dietary supply of such acids would mobilize the PUFA from the depots. In the present study the relative amount of PUFA in the PFA and to some extent also in the GFA gradually decreases in the lipoprotein fractions. It can at present not be explained why arachidonic acid would exert this effect. It can not be ruled out that some part of the decrease is caused by losses during the laboratory handling of the specimens although this seems unlikely as the low values have been obtained in different series of analyses.

Summary

1 Ethyl arachidonate was given to two male patients with cardiosclerosis and elevated blood lipids. Both showed lower lipid values when 20 g was given daily but only one reacted to a dose of 5 g daily

2 The tetraene content of the different serum fractions showed a rise during the administration of arachidonate. This is most evident in the phospholipids and least apparent in the glycerides.

3 The diene content of the lipid fractions showed a simultaneous decrease.

4 The changes were most evident on a dosage of 20 g daily but persisted to a certain extent also on prolonged medication of 5 g daily

5 A possible explanation of these findings has been outlined

Acknowledgements

Generous amounts of ethyl arachidonate were put t disposal by F Hoffman La Roche and Co. Thanks are due to Mr Kent Hammarstrand for skilful technical assistance

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Primary (Idiopathic) Auto-immune Haemolytic Anaemia

By

AAGE VIDEBÆK

Auto-immune haemolytic anaemia (A. I. H. A.) is taken to mean an acquired haemolytic anaemia with antibodies detectable on the patient's own red cells in vitro. These antibodies are generally assumed to play a role also in vivo in the hyperhaemolytic condition. This criterion, therefore, rules out patients in whom no erythrocyt sensitization has been demonstrated, despite the laboratory and clinical findings otherwise identical with those in patients with primary A. I. H. A. with demonstrable erythrocyt antibodies. It is reasonable to believe that the patients thus excluded also suffer from erythrocyte sensitization. Only it has not been demonstrable by the technique used.

Present series

This paper and two others (17, 17 a) will report on 41 consecutive cases of haemolytic anaemia observed during the period 1951—1961. All had positive Coombs test. The patients were seen regularly in the Out-patient Department, a number of them for years, and several have been repeatedly admitted as in-patients.

According to table I the auto-immune haemolysis must be considered secondary, i. e. a complication to some other disease, in approx. 90 (36 out of 41 patients). Only 5 were classified as primary A. I. H. A. This much smaller group comprises residual cases which could not be related to any underlying disease, even after thorough investigation and a long follow-up period.

Diagnosis

The diagnosis of the haemolytic condition, which is usually accompanied by anaemia, was in many instances obvious. This applied when the anaemia had set in suddenly, unpreceded by loss of blood, and co-existed with latent or manifest jaundice, marked reticulocytosis, and a hyperactive erythropoiesis. Other cases were less obvious, encountered especially in patients with simultaneous relative insufficiency of the red cell production and thus with less marked jaundice and reticulocytosis. In such instances the diagnosis haemolytic syndrome has been based

Submitted for publication October 4, 1961

Table I Classification of 41 cases of auto-immune haemolytic anaemia

	M	F	Total
Secondary			
S. L. E.	0	10	10
Malignant systemic diseases	12	7	19
Others	4	3	7
	16	20	36
Apparently primary	2	3	5
Total	18	23	41

on the observation of the MCV and MCHC of the red cells, their appearance and osmotic fragility often combined with investigation of the sternal marrow and with determination of the serum iron, haptoglobin and the life span of the red cells That the condition was inter pretable as sensitization of the red cells was indicated by a positive outcome of Coombs direct test carried out in the usual way These investigations have lately been performed at the Blood Bank of the University Hospital (E Freiesleben) previously at the State Serum Institute (V Friedenreich) The Coombs serum used throughout the 10-year period was very potent, and regard was paid to the prozone phenomenon It was not until recent years that conscious efforts were made to include the non-γ component, but very probably it was present also in the serum used originally

Since erythrocyte sensitization is inter pretable as one of several possible signs of auto-immunization the diagnosis could almost always be supported by the demonstration of other signs of immunization, such as a highly elevated E. S R a false positive W R. an elevated concentration of one or more immune antibodies, positive "rheuma tests etc. In a few cases leukocyte and platelet agglutinins were also observed.

After A. I H. A. had been diagnosed, a thorough procedure for general diagnosis and often many years continuous follow-up were used in order to arrive at a more detailed clinical classification. According to this classification the number of patients with apparently primary A.

Table II Some laboratory findings in 5 cases of apparently primary auto-immune haemolytic anaemia

Case	Sex	Age	Observation period (years)	Strength of Coombs direct test	Cold agglutinin titre	E. S. R. (mm)	WR	Serum protein, g 100 ml: Total	Albumin	α_1-globulin	α_2-globulin	β-globulin	γ-globulin	Serum iron $\mu g/100$ ml	Osmotic fragility conc. of NaCl at incipient and total haemolysis	Fig. nos.
1	M	10	7.5	++++	4	100	neg	7 6	4 1	0.30	0.53	1 02	1.67	135	0 85–0.20	1 2
2	M	63	4.5	+++	128	142	neg	6.5	4.8	0 47		0.68	0 52	253	0.85–0.20	3
3	F	35	8	+	32	110	+	6 2	4.9		0.23	0.30	0.76	126	0.50–0.36	4
4	F	55	2.5	+++	4	123	neg.	6.2	4 1	0 45	0.38	0.76	0.53	67	0.60–0.30	5
5	F	67	2	+++	8	102	neg	6.1	3 1	0.46	0 49	0.70	1.32	112	?	6

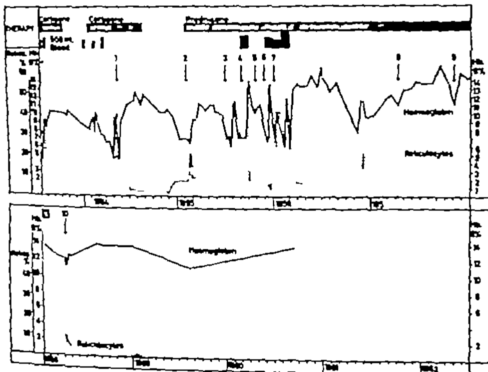


Fig. 1 Case 1 Haemoglobin level, treatment, and crises in boy with acquired haemolytic anaemia. Throughout the observation period, so far of 7 years, the Coombs direct test has been strongly positive. The figures indicate crises which occurred in connection with (1) pneumonia, (2) rubella, (3) excess. sport, (4) acute gastroenteritis, (5) distortion of the ankle (6) excessive sport, (7) catarrhal fever (8) acute gastroenteritis, (9) Asiatic flu, (10 catarrhal fever (Cf. table I)

I. H. A. is so small that this variety must be considered fairly uncommon embracing at most 5 patients or only approx. 12 of all the patients. Therefore, the tabular survey (table II) will be supplemented by the histories of each of these 5 patients.

Case 1 No family history of blood diseases. His father had diabetes. At the age of 10 (1953) the patient developed febrile disease attended by jaundice and sudden anaemia. The stools were of normal colour. During bed rest the jaundice disappeared. Since then, intermittent episodes of jaundice until, 2 months later he was admitted with a haemoglobin level of 4.4 g/100 ml, reticulocytes 5.4

E. S. R. 100 mm, W. B. C. 10,200 normal differential count, platelet count 172 000. In the cellular sternal marrow 68 were normoblasts. Serum bilirubin 2.0 mg/100 ml, increased osmotic fragility and high serum iron. Spleen just palpable (cf. table II)

After treatment (cf. fig. 1) with 2 blood transfusions and cortisone splenectomy was carried out in July 1953 (micr. exam. showed mild fibrosis) The haemolytic crisis subsided. Three months later cortisone was discontinued, but now the anaemia became worse. Only when blood transfusions and cortisone were resumed could the haemolysis be controlled, apart from acute exacerbation during an attack of pneumonia. After having been resumed for 7 months, cortisone was again discontinued, but the patient slowly developed severe anaemia and crises when he had

Table I Classification of 41 cases of auto-immune haemolytic anaemia

	M	F	Total
Secondary			
S. L. E.	0	10	10
Malignant systemic diseases	12	7	19
Others	4	3	7
	16	20	36
Apparently primary	2	3	5
Total	18	23	41

on the observation of the MCV and MCHC of the red cells their appearance and osmotic fragility often combined with investigation of the sternal marrow and with determination of the serum iron haptoglobin and the life span of the red cells. That the *condition was* inter pretable as sensitization of the red cells was indicated by a positive outcome of Coombs direct test carried out in the usual way These investigations have lately been performed at the Blood Bank of the University Hospital (E. Freiesleben) previously at the State Serum Institute (V Friedenreich) The Coombs serum used throughout the 10-year period was very potent, and regard was paid to the prozone phenomenon It was not until recent years that conscious efforts were made to include the non-γ component, but very probably it was present also in the serum used originally

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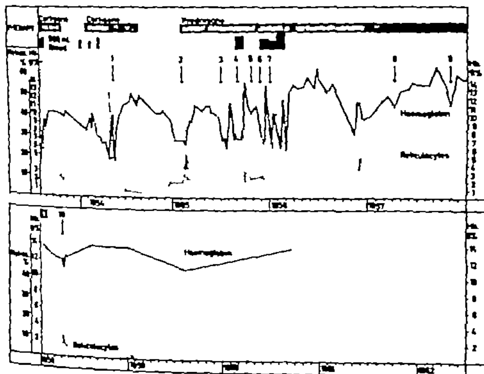


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Fig 2. Case 1 Right hip joint in 15-year-old boy with severe auto-immune haemolysis of 5 years duration. The hip joint changes had developed gradually setting in 2 1/2 years after the haemolytic symptoms had started.

rubella. Although he was treated during the subsequent 3 1/4 years with prednisone in varying doses (5 to 40 mg daily) he had repeated haemolytic crises. Every time, they could be controlled by raising the dosage of corticosteroid. On a few occasions blood transfusions were given as well Twice a crisis appeared to have been elicited by physical exertion (sport) once by a distortion, 6 times by infection (cf. fig 1) Twice the cause of the crisis was unknown. Since January when he was 14 the patient has not received corticosteroids and has not had any typical crises. On rare occasions he has exhibited mild jaundice with somewhat darkened urin The above mentioned crises have occasionally been accompanied by pain below both costal borders, but cholecystography in August 1958 showed normal appearances.

In December 1955 the boy developed pain in his right hip. There was some contracture and tenderness, but X rays of the pelvic bones and hip joints showed no abnormalities. The pain soon yielded to bed rest. In May 1956, when the patient was 13 a flexion contracture of the right hip joint was noted with 2 cm shortening of the right leg and a scraping sound on movement of the hip joint. Now X-rays revealed flattening of the head of the femur and an increased width of the joint space. In May 1956 also patchy sclerosing but no fracture. During the next 18 months the joint was spared by the use of a Thomas splint and English canes. During the first months, the bony structure around the joint line grew denser and in August 1956 patches of destruction were observed. After October 1957 two years after the onset of symptoms, the condition was stationary according to the X ray films. The epiphysis was practically unaffected (fig 2) In February 1959 when the boy was 16, the lesion had healed, the head of the femur being flattened and somewhat patchy During the entire follow-up period the left hip joint had been normal.

B. C. G vaccination had been carried out in early childhood The Mantoux test is positive, and chest radiography revealed normal conditions. To be on the safe side, cultures from the gastric lavage and urine were done but without showing any growth of tubercle bacilli.

The boy has now been followed closely for more than 7 years. He entered puberty at the normal time and at 17 his height is 180 cm. He is a tailor's apprentice and has not had a day's illness during his apprenticeship.

Throughout the entire follow-up period the Coombs direct test, performed about twice a year has been strongly positive, even in periods where the haemolysis has been fully compensated, and the reticulocyte count only moderately elevated The platelet count has always been normal The "leukocyt count" has often been high during crises, as a rule 30,000—40 000 a maximum of 84 000, but then 50—80 of the cells have been normoblasts, so in actual fact the leukocytosis has been slight.

The E. S. R. has also been followed. During the crises it has been greatly elevated, but in the intervals normal The β and γ globulins were elevated, but no L. E. cells could be demonstrated.

The investigations have not shown haemolysin or an elevated cold agglutinin titre but increased osmotic fragility

Summary

A 10-year-old boy suddenly developed severe A. I H A. which has been followed ever since, or for 7 1/2 years. Splenec

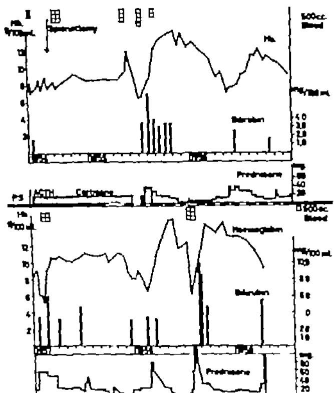


Fig 3. Case 2. Variations in haemoglobin and serum bilirubin during the course and treatment in 63-year-old man with primary auto-immune haemolytic anaemia (Cf. table II)

tomy was ineffective, but cortisone — and later prednisone — proved of great therapeutic value. After having been continued for 4 1/2 years, however the medication could be gradually discontinued. During these years, the patient had several crises, and after the disease had persisted for 5 1/2 years he developed a peculiar process in his right hip joint, leading to flattening of the head of th femur sclerosing, and shortening of the leg. This complication ran its course for pproximately 3 years. During the past 3 years corticosteroid has not been needed. The haemoglobin level is now virtually normal, but an elevated retic ulocyte count and mild jaundice persist, and head colds are accompanied by darkening of the urine.

Case 2 A 62-year-old man who had been suffering from lumbago for many years. In other respects he had been in good health, until 1954 when he became anaemic, having Hb. level around 8 g/100 ml (fig 3) greatly elevated E. S. R., hyperactive bone marrow completely dominated by normoblasts, and in addition mild jaundice (table II) Treatment with ACTH had little effect. I August 1954 therefore splenectomy was done, but without any particular improvement of the anaemia For some time there were 5—6 reticulum cells in the blood, and throughout the period of the disease numerous normoblasts, 15—70 of "leukocyte count which ranged from 10,000 to 90,000 The platelet count alw y exceeded 100,000, and the reticulocyte coun was usually 10—20

Throughout the observation period (about 5 years) the patien needed corticosteroid in arying doses and could hardly manage on less than 20 mg prednisone day Several

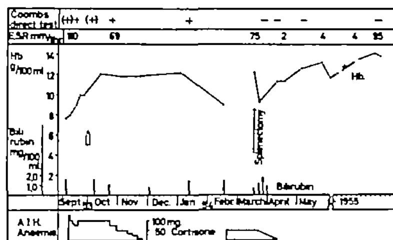


Fig. 4. Case 3. Haemoglobin, serum bilirubin, E. S. R., and Coombs direct test during treatment with cortisone and following splenectomy in case of auto-immune haemolytic anaemia in a young woman.

crises occurred, especially in the event of infection (febrile bronchitis, urinary tract infections, gastroenteritis, epididymitis). The crises could be overcome by high doses of prednisone, and a few times blood transfusions were required. Febrile transfusion reactions often followed but not when washed erythrocytes had been used. The main complaint was severe cramps in the leg muscles through all the years, frequent headaches, and gradually a chronic urinary infection.

In March 1958 the serum iron was 0.071—0.029 mg/100 ml as compared with the original value of 0.200 mg/100 ml, and oral iron medication effected a marked increase in the haemoglobin level. Periodically the patient had haemoglobinuria.

In August 1958 a crisis occurred, the serum bilirubin values being up to 40.9 mg/100 ml, glutamic-oxalacetic acid and glutamic pyruvic acid transaminases elevated, prothrombin time reduced, and alkaline phosphatase elevated. This was presumably inoculation hepatitis.

The patient went on working until December 1958, but after that time he was constantly troubled by fatigue, pain in his legs, a tendency to oedema, frequent urinary infections, exertional dyspnoea, cough, fever and gradually increasing drowsiness, and he died in shock.

During the entire observation period Coombs direct test was positive. The E. S. R. was high during the crises, but otherwise normal.

Autopsy (University Institute of Pathological Anatomy). Bone marrow in the vertebral column dark red to black, and the same applied to the sternal marrow. The lungs were firm, dark, and the heart hypertrophic. Liver smooth, firm, dark on cut surface measuring 10 × 26 × 30 cm. In the lungs thickened alveolar septa with numerous macrophages holding haemosiderin. In the kidneys also pronounced haemosiderin deposits. On the other hand, no deposition of iron worth mentioning in the bone marrow of the femur or in the skin.

Summary

A 62 year-old man developed classical A. I. H. A. He was closely observed for 4 1/2 years. Splenectomy was of no avail. Constant treatment with cortisone or prednisone was essential. Coombs test was always positive, but it was only during the crises that the E. S. R. was elevated. Jaundice was present all the time, but varied in degree. A considerable iron deficiency presumably due to haemoglobinuria, was successfully treated with iron medication. Death occurred, after frequent infections, because of pulmonary insufficiency. Autopsy revealed hyperactive erythropoiesis, hepatomegaly and haemosiderosis of the liver, lungs, and kidneys, but no excess iron deposition.

Case 3. In 1935 when the patient was 17 years of age, a routine investigation revealed a false positive Wassermann reaction.

In 1953 she was admitted with severe

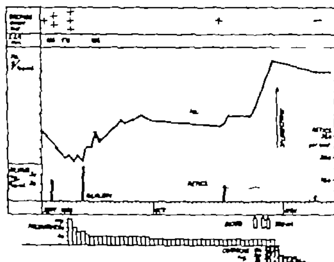


Fig. 5 Case 4. Result of treatment with prednisone and subsequent splenectomy in case of apparently primary auto-immune haemolytic anaemia which lasted only for few months.

anaemia (cf. table II and fig. 4) but no normoblasts in the blood, reticulocyte values of 5—10 %, W. B. C. about 11,000 platelets 150,000—200,000, slight splenomegaly nor and cholecystography. Gynaecological examination disclosed uterine fibromatosis. N articular symptoms, pyrexia, or exanthema.

Cortisone treatment was instituted, 100 mg a day. This resulted in a remission which was maintained for about 2 months after cortisone was discontinued. Then, under cortisone cover splenectomy was performed in March 1953. The spleen measured 25 × 16 × 9 cm. Microscopic examination showed small, scattered lymph follicles, occasional leukocytes and plasma cells, and vascular lumina distended with blood. After the operation Coombs direct test was negative, while before it had been positive. Although the prednisone was rapidly levelled off, the Hb. went on rising up to the normal range during the subsequent months. However the E. S. R. was low for only about year. A weak cold agglutinin was found in the blood, just as befor the operation.

As recently as March 1961 efforts were made to persuade the patient to attend out patient follow-up, but in vain. She is well and orking in laundry.

Summary

A 35-year-old woman, with a history of positive W. R. 18 years previously suddenly developed A. I. H. A. which soon could be controlled by cortisone. When cortisone was withdrawn she had a recurrence and the somewhat enlarged spleen was removed. She has been working since the operation. Coombs direct test became negative after the splenectomy but a cold agglutin n of a low titre persists.

Case 4. A 55-year-old woman with a history of Graves disease t the age of 37. At that time she had been subjected to thyroidectomy developed myxoedema and had been treated with desiccated thyroid ever since.

In July 1958, when she was 55 she suddenly became anaemic. She was admitted to local hospital where she was given 5 transfusions.

When she entered the University Hospital in September 1958 she was deeply anaemic, jaundiced, and running low-grade fever. Her stools were of normal colour the urine dark (cf. also table II). Reticulocyt count 6—16.2 per cent, haptoglobin 0, platelets approx. 90 000, W. B. C. approx. 30,000, no normoblasts in the blood.

On prednisone, 120—40 mg daily (cf. fig. 5) the general condition promptly improved. In the course of few days the Hb. rose and despite an initial Hb. value of 4.6 g/100 ml, no blood transfusions were needed. The patient was discharged to her home in early

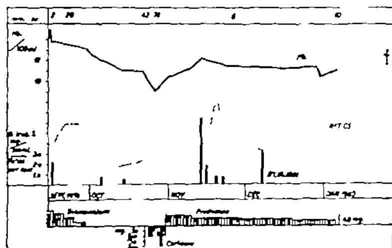


Fig 6. Case 5. Treatment and variations in haemoglobin, reticulocytes, and serum bilirubin during the last months of auto-immune haemolytic anaemia which had been diagnosed 2 years previously The high bilirubin in the middle of November coincided with a high haemoglobin value as well as a low reticulocyte count and was due to biliary tract occlusion.

October but on re-admission 3 weeks later she still showed marked reticulocytosis, mild jaundice, and severe anaemia. After three pints of blood she had splenectomy under cortisone cover" The spleen weighed 390 g and microscopic examination did not show any unexpected changes. Within 10 days the steroid medication was withdrawn During the 2 1/2 years which have elapsed since the operation the patient has been in good health with a Hb. level of 14—15 g/100 ml.

Summary

A 55-year-old woman with severe A. I H A (positive Coombs test, no cold agglutinins) improved considerably on prednisone, but after removal of the spleen Coombs test turned negative and the prednisone therapy could be discontinued She has since remained well for 2 1/2 years.

Case 5. An elderly woman who had been troubled, from her youth by a large number of infections (otitis in 1914 urinary tract infection in 1920, salpingitis in 1929 pneumonia in 1945 paralytic poliomyelitis in 1952, pulmonary infection in 1956)

In 1957 at the age of 67 she developed anaemia (cf. table II and fig 6) W B. C. 5 000—10,000 no normoblasts in the blood, platelets 200 000—300,000. Despite treatment with prednisone and triamcinolone the E. S. R. remained high and considerable haemolysis persisted Gradually her appearance was very much Cushingoid.

In September 1959 she was admitted to the University Hospital with severe pain in her back. Pneumonia, pulmonary infarction, and a urinary tract infection were found besides compression fracture of 4 vertebral bodies complicated by abdominal herpes zoster It was felt that the corticosteroid dosage had to be lowered, but this resulted in a steady decrease in Hb. (fig 6) and an increase in E. S. R. Pneumonia towards the end of October gave rise to adrenocortical insufficiency A supplement of cortisone soon controlled the Hb. Treatment was continued with prednisone, together with anabolic steroid (Durabolin) Within a fortnight the Hb. rose from 9 0—12.2 g/100 ml. In the middle of November cholangitis with short-lasting manifest jaundice. The serum bilirubin rose to 6 4 mg/100 ml, but the Hb rose and the reticulocyte count was fairly low The patient was now discharged but was admitted 4 weeks later as an emergency to another hospital. She was in shock and died showing signs of hepati insufficiency

Autopsy (Institute of Pathology Frederiksberg Hospital) showed marked dilatation of th entire biliary system due to severe contriction of Vater s papilla (micr exam. fibrosis) Several stones in the gallbladder Spleen and li er large and ery dark.

Microscopic examination revealed haemosiderosis of the spleen, severe hyperplasia of the bone marrow and intrahepatic pericholangitis, but no signs of systemic lupus erythematosus.

Summary

A 57 year-old woman who had been troubled by infections all her life developed severe A. I. H. A. with a positive Coombs test without the presence of cold agglutinins. On triamcinolone and later on prednisone, the Hb. level could be kept high, but she suffered compression fractures of several vertebral bodies. Corticosteroid was tentatively withdrawn, but had to be resumed. After that, the Hb. could be maintained on a reasonable level. She died in shock, and autopsy showed hyperplasia of the bone marrow cholelithiasis, and severe cholangitis.

Discussion

Classification

It is remarkable that in the author's material, the primary form of A. I. H. A. occurred in only 12 % of the patients with auto-immune haemolysis (5 out of 41). In previous series (4 5 8 12) the primary variety has been by far the most common. For example, Crosby & Rappaport (4) found that 60 of 57 cases of I. A. H. A. were idiopathic. Among 131 patients with A. I. H. A. Dacie (5) found 93 or 70 to be of the primary type. Dausset, in summary diagnostic report on 128 patients with A. I. H. A. (8) classified not less than 93 as idiopathic (73 %). In a previous Danish series (12) 11 out of 18 cases were idiopathic. In our series however 10 patients (or 24) had classical S. L. E. (17) from the outset or at a later stage, while in Dacie series the S. L. E. group comprised only 5 patients or 3.8 %. In Dausset series A. I. H. A. is stated t be secondary to S. L. E. in only 2 cases or 1.6 of the whole series. When t is considered that A. I. H. A. was the initial sign in 5 out of our 10 patients with S. L. E., that in some cases it was years before the diagnosis of S. L. E. became obvious (17) and that it may be long before an underlying malignant systemic disease or tumour is disclosed (4) the importance of a painstaking history and investigation is clear. In particular constant follow-up is decisive for clinical classification. In this respect, however the materials from the different clinics are not quite comparable. In our series 19 patients, or 46 were found to belong to the group of malignant systemic diseases (table I) which comprised only 18 or 14 % of Dacie's cases and 28 or 25 % of Dausset's. If the finding in our series, that at least one-quarter of all patients with chronic lymphatic leukaemia develop A. I. H. A. (16) and that approx. 40 % of the patients with S. L. E. do so (17) holds universally it is likely that a number of S. L. E. cases are concealed among Dacie's and Dausset's large number of "idiopathic" A. I. H. A. The fairly large number of cases which in our series were secondary to malignant systemic diseases of course decreases the percentage of primary cases.

Clinical findings

Clinically the condition in the active phase is characterized by haemolytic jaundice and in some cases by severe anaemia, often of a sudden onset. Just as in hereditary spherocytosis, crises are common, perhaps mainly in the winter (4). They may be elicited by stress, particularly infections and physical exertion or trauma (cf. int. al. fig 1). We did not observe aplastic crises in the primary form of A. I. H. A., but a few cases have been reported in the literature (int. al. 9 13). Reticulocytopenia has been observed during the crises in quite a number of patients (3). During latent phases of

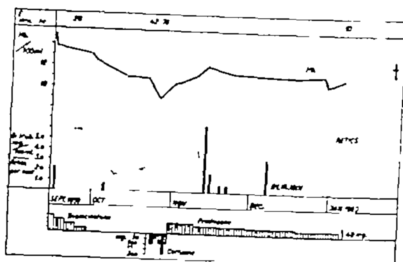


Fig. 6. Case 5. Treatment and variations in haemoglobin, reticulocytes, and serum bilirubin during the last months of auto-immune haemolytic anaemia which had been diagnosed 2 years previously. The high bilirubin in the middle of November coincided with a high haemoglobin value as well as a low reticulocyte count and was due to biliary tract occlusion.

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Microscopic examination revealed haemosiderosis of the spleen, severe hyperplasia of the bone marrow and intrahepatic pericholangitis, but no signs of systemic lupus erythematosus.

R. and in some cases by elevated serum concentrations of the immune globulins. However only one of our patients had a false positive Wassermann reaction. She was a young woman with a fairly mild immune haemolysis, and possibly she will later turn out to have S. L. E.

In all 5 cases Coombs' direct test was positive, as a rule strongly positive. Furthermore, the cold agglutinin titre was slightly elevated in one (case 2). During the entire observation period, Coombs' test was performed at short intervals. In cases 1, 2, and 5 it was strongly positive through 7 1/2, 4 1/2 and 2 years, even during the periods of several years when the hyperhaemolysis was well-compensated, that is to say the Hb. might be normal while other signs indicated increased haemolysis. In the patient who has not yet received any treatment the E. S. R. was constantly above 100 mm, while it returned to normal in cases of effective treatment, although the Coombs' test might remain strongly positive. Case 3 had a weak, but indubitably positive Coombs' test through 6 months, but after prednisone medication and splenectomy the Coombs' direct test became negative, whereas the cold agglutinin titre remained slightly elevated (fig. 4) and the E. S. R. which had become normal after the splenectomy rose again later on. Following splenectomy and prolonged prednisone medication, case 4 had a negative Coombs' test (cf. fig. 5). This patient has not relapsed after 2 1/2 years' follow-up period.

In other words, while a positive Coombs' direct test in these cases was a sure sign of manifest or latent haemolysis, hyperhaemolysis could not be ruled out even if the Coombs' test became negative during the course. When the E. S. R. was high during the course of A. I. H. A., the hyperhaemolysis was usually pronounced and decompensated. Thus, the E. S. R. was, on the whole, a more useful guide than Coombs' test in the evaluation of A. I. H. A.

Course and prognosis

Out of the 5 patients with primary A. I. H. A., 2 died (cases 2 and 5) after 4 1/2 and 2 years' illness respectively. Case 2 succumbed to cardiac-pulmonary insufficiency as a direct consequence of the disease, while case 5 died of relative adrenocortical insufficiency caused by corticosteroid medication which, however, he could not do without.

Case 1 is alive and well after having had hyperhaemolysis for 7 or 8 years. During the first 4 or 5 years the course was far more dramatic (fig. 1) than in the last 3 years, during which no treatment has been needed, since there have been no crises and the Hb. has been near normal. Case 3 had haemolytic anaemia at least for 18 months, but after that no signs. Case 4 is alive and working without anaemia for the past 2 1/2 years, after a period of haemolysis lasting for only 3 months.

Among the 5 cases the haemolytic condition in one may perhaps have been secondary to S. L. E. Three (2 of whom died) had chronic haemolysis, while in one case the haemolytic anaemia must be considered as subacute and benign.

The literature does not contain much concrete information on the course of primary A. I. H. A. of several years' duration.

Young and Miller (18) have reported 5 cases followed for from 4 to 7 years after the introduction of Coombs' test. All of them had previously been splenectomized. In one case they observed 6 crises, and the Coombs' test remained positive

chronic A. I H. A the condition may be characterized merely by mild scleral jaundice and periodical choluria while the Hb may be normal or only very slightly lowered.

The blood picture was that which is so well known but a man with chronic severe haemolysis (case 2) developed severe iron deficiency evidently due to haemoglobinuria. It might then be worth investigating whether patients with a long history of severe haemolytic syndrome have developed iron deficiency In our case a striking response to iron medication was observed None of the patients had leukopenia. Only case 4 had thrombocytopenia, but of no practical significance.

Case 1 developed a strange, deforming and sclerosing lesion in the head of the femur (fig 2) resembling changes which have been interpreted as aseptic necrosis of bone, likewise in the head of the femur as observed int. al in 6 out of 39 patients with sickle-cell anaemia (11) All six were males ranging in age from 13 to 31 years. The changes were bilateral in three. Similar changes were described as early as 1943 in sickle-cell anaemia (1) but have later been observed in several other conditions (14) likewise entailing defects in combined haemoglobin especially Hb S/Hb. C disease (15) In our case, the X-ray appearances corresponded closely to those described. It is no less remarkable that corresponding changes have been found in a few patients with S L. E Thus 9 out of 400 patients with S L. E. had aseptic necrosis of bone in the head of the femur bilateral in 8 and unilateral in one (9 a) Dubois believed that the changes were conditioned by a special localization of the S L. E. but he did not relate the bony changes to haemolytic anaemia. Two of his patients, however had haemoglobin values of 10.7 and 8 g/100 ml, reported as unexplained anaemia. Our case 1 with "primary A. I. H. A and necrosis of bone may have a so far not obvious S L. E. the necrosis being a complication to intravascular haemolysis or erythrocyte aggregation or due to arterial thrombosis of a different pathogenesis. Thrombosis is a not infrequent complication to S L. E. and primary A. I H. A.

Ulcer of the leg did not occur in any of our cases. Cholelithiasis is said to be not particularly common (8) Accordingly out of the two fatal cases only case 5 had several faceted pigmented gallstones and moreover severe dilatation of the biliary tree due to fibrosis of the sphincter of Oddi, while case 2 did not have gallstones after having been suffering from severe haemolysis for 4 years. Cholecystography performed in cases 1 and 3 after severe haemolysis of 5 years and 6 months duration respectively showed normal conditions.

Investigations for haemolysis

The osmotic fragility of the red cells may be considerably increased, as in our cases 1 and 2 and the red blood picture may be characterized by spherocytes. This situation is not however likely to be mistaken for congenital spherocytosis, since in auto-immune haemolysis a number of red cells are visibly undergoing lysis i e showing up as ill-defined, large and pale cells unlike the sharply defined cells of congenital haemolytic anaemia The high E. S R gives a useful differential diagnostic hint. In uncomplicated hereditary haemolytic anaemia the E S R is normal or low and Coombs test negative unlike the auto-immune haemolytic conditions which are usually accompanied by greatly increased E. S

producing cells (reticulum and plasma cells)

Perhaps the outcome of splenectomy may be predicted as favourable in cases where Cr^{51} labelling has revealed a particularly brisk activity over the spleen (indicating a severe destruction of the red cells in this organ) but not over the liver (indicating that intravascular haemolysis is slight) (6) This aspect has not yet been sufficiently clarified and these tests were not performed in our series. The size of the spleen and its microscopic appearances do not appear to be reliable indicators of the effect of splenectomy Possibly the results are poorer in the most severe cases and in patients younger than 40 (2) Chertkow & Dacie came to the conclusion who that a poor response to corticoid medication does not militate against a possibly favourable result of splenectomy This conclusion is supported and aptly illustrated by the therapeutic result in our case 4 (cf fig 5)

As reported under case 2, iron medication was effective for a time, when iron deficiency had arisen presumably due to prolonged haemoglobinuria.

Blood transfusions were kept to a minimum, but could not always be avoided in the event of crises and splenectomy In several haemolytic crises, however we managed merely by raising the dosage of prednisone. Two patients (cases 1 and 2) developed hepatitis, presumably as homologous serum jaundice Patients with A. I H. A. appear to be very apt to form isoantibodies following transfusion and in particular specific auto-antibodies may be formed, evidently as link in the auto-immunization. These auto-antibodies belong mainly to the Rh group, but others have been found as well (int. al. 10) This, of course, has to be considered carefully in selecting donors. Among our 5 patients, one (case 5) showed anti-Kell as well as anti-c. Since anti-c, apart from being present free in the patient's serum, could be eluted from her red cells, it is presumably an auto-antibody (Dr K. Gert Jensen, the Hospital Blood Bank)

Summary and conclusion

In the author's opinion primary auto-immune haemolytic anaemia (A. I H. A.) is fairly uncommon. Careful investigation and even several years observation are needed to rule out underlying diseases, primarily systemic lupus erythematosus (S. L. E.)

Among 41 cases of A. I H. A. seen during a period of 10 years, 5 were idiopathic. Their case histories are reported.

One developed aseptic unilateral necrosis of the head of the femur This has previously been considered peculiar to some congenital haemolytic anaemias. One developed severe iron deficiency presumably because of prolonged haemoglobinuria. One was perhaps suffering from S. L. E

The course in 4 cases was chronic, with frequent haemolytic crises. Unlike congenital spherocytosis, the erythrocyte sedimentation rate in untreated cases was always greatly elevated.

Coombs direct test was constantly positive in the 3 chronic cases, even when the treatment resulted in remission and the E. S R. returned to normal The Coombs test was never positive without simultaneous hyperhaemolysis, but the increased red cell destruction might be fully compensated and the Hb. therefore normal. On the other hand, Coombs direct test was not positive in all periods of haemolytic anaemia, and the E. S R.

through 4 years In another case only one crisis occurred although Coombs test had been positive for 18 months, but then became negative without any treatment other than splenectomy one year previously In a third case Coombs test was positive through 5 years during which the Hb was normal, but then the Coombs test spontaneously became negative. Thrombo-embolic phenomena are said to be common (18) even in thrombocytopenic patients (4) As is so often the case, it appears to be difficult to predict the prognosis in each individual case. Several authors have reported low reticulocyte counts and thrombocytopenia especially associated with purpura, as poor prognostic signs (4 8) In particular they have stressed the presence of free antibodies in the plasma, manifesting itself as a positive indirect Coombs test (8)

Treatment

Prednisone was the main therapeutic agent. In cases 2 and 5 the medication has been almost permanent for several years, attempts at discontinuation immediately resulting in increased haemolytic activity Case 1 also had to be treated for a long time, but after 4 years treatment he has managed without treatment for 3 years, although he has not been cured In cases 3 and 4 the steroid medication could be withdrawn after splenectomy Improvement has been observed immediately or a few weeks after the commencement of the treatment. The dosage will be seen from the figures Thus primary A. I H A. does not always require permanent corticosteroid therapy but in certain periods it appears to be of decisive significance, sparing the patient transfusions, and the value of the long term treatment must, therefore, be considered to outweigh its risk. That the risk is great is well known and apparent also from the present series. Case 2 developed a typical Cushing appearance and repeated urinary tract infections. Case 5 also developed a typical Cushing appearance, had several fractures of the spine, and died of acute, drug induced adrenocortical insufficiency in connection with a biliary tract infection.

In the course of the treatment, therefore withdrawal of the corticosteroid should be tried. If the patient cannot do without it, he should be kept under close supervision, and splenectomy should be contemplated, especially in cases which require high doses of corticosteroid for many months. Dameshek & Komninos (7) splenectomized 9 patients, and this resulted in complete remission in 6. They do not state, however the length of the remission or whether their patients had idiopathic or secondary auto-immune haemolysis.

Four of our patients had splenectomy two (cases 1 and 2) as primary treatment and definitely without effect. In cases 3 and 4 on the other hand the splenectomy was followed by improvement, the Hb level being perceptibly increased and the Coombs direct test becoming negative. In case 3 the E. S R remained low for a year but then rose again. As is apparent from fig 4 however corticoids were administered for a short time before and after the operation. Case 4 had been treated ineffectively with prednisone for 7 weeks. After splenectomy Coombs direct test became negative the haemoglobin level returned to normal and has remained so for 2 1/2 years. In this case, therefore the splenectomy seems to have been beneficial, although the spleen weighed only 370 g and did not show any marked hyperplasia of the antibody

producing cells (reticulum and plasma cells)

Perhaps the outcome of splenectomy may be predicted as favourable in cases where Cr^{51} labelling has revealed a particularly brisk activity over the spleen (indicating a severe destruction of the red cells in this organ) but not over the liver (indicating that intravascular haemolysis is slight) (6). This aspect has not yet been sufficiently clarified, and these tests were not performed in our series. The size of the spleen and its microscopic appearances do not appear to be reliable indicators of the effect of splenectomy. Possibly the results are poorer in the most severe cases and in patients younger than 40 (2). Chertkow & Dacie came to the conclusion also that a poor response to corticoid medication does not militate against a possibly favourable result of splenectomy. This conclusion is supported and aptly illustrated by the therapeutic result in our case 4 (cf fig 5)

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is therefore considered to be a more useful guide in the treatment. The basic treatment was corticosteroid medication, but one patient died presumably because of this treatment, in shock and with complicating cholangitis. Another patient died of recurrent infections and pulmonary insufficiency which was presumed to be due in part to haemosiderosis.

In one case permanent corticosteroid therapy was needed while in another case it could be discontinued at the end of 4 years although at the end of another 3 years the patient still showed a latent haemolytic condition with a positive Coombs test.

Splenectomy was carried out in 4 cases, but was ineffective in 2 of them. One patient evidently was cured after splenectomy and one considerably improved.

Clinically chronic A. I. H. A. closely resembles hereditary spherocytosis, but apart from a few acute cases, the prognosis is far more serious.

Acknowledgement

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Auto-immune Haemolytic Anaemia in some Malignant Systemic Diseases

By

Aage Videbæk

It is known that auto-immune haemolytic anaemia (A. I. H. A.) may arise in the course of a large variety of diseases. This applies particularly to malignant systemic diseases, collagen diseases, various infections, and a number of malignant as well as a few benign tumours. With few exceptions, however, this complication is so rare that it must be regarded extraordinary and therefore uncharacteristic of the basic disease. The main exceptions are systemic lupus erythematosus (S. L. E.) and chronic lymphatic leukaemia. In these two conditions, however A. I. H. A. is so common a complication that it must be considered an important sign.

The following is an account of our experience of A. I. H. A. in patients with malignant systemic diseases who have been under constant supervision during the period 1951—1960. The criteria of A. I. H. A. have been a haemolytic syndrome combined with a positive direct Coombs' test.

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I. Chronic lymphatic leukaemia

The series comprises 13 patients 12 of whom were found among 47 consecutive cases of chronic lymphatic leukaemia.

Fig. 1 illustrates the occurrence of phases of haemolytic anaemia in these patients (9 males and 4 females). The age at which chronic lymphatic leukaemia was diagnosed ranged from 48 to 89. As is apparent from the table the complication A. I. H. A. may be among the very first signs (5 cases) or occur at any stage of the course, even up to 12 years after the diagnosis of leukaemia (case 12). It will be seen also that the haemolytic phase may be of a few weeks duration or a more constant feature, lasting for up to 2 or 3 years (case 5) but as a rule for 12 to 18 months. A couple of patients had two or three haemolytic phases, while the majority had only one. In some cases the haemolytic complication was an outstanding feature of the symptomatology of the leukaemia, while in others it was short-lasting (cases 3, 6, 10 and 12)

is therefore considered to be a more useful guide in the treatment. The basic treatment was corticosteroid medication but one patient died presumably because of this treatment, in shock and with complicating cholangitis. Another patient died of recurrent infections and pulmonary insufficiency which was presumed to be due in part to haemosiderosis

In one case permanent corticosteroid therapy was needed, while in another case it could be discontinued at the end of 4 years although at the end of another 3 years the patient still showed a latent haemolytic condition with a positive Coombs test

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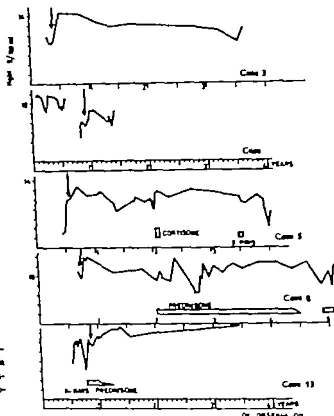


Fig. 2. Variations in haemoglobin following splenectomy in 5 cases of chronic lymphatic leukaemia with auto-immune haemolytic anaemia.

unusual. At the same time there might be latent or manifest jaundice, a high reticulocyte count, and fever. Not infrequently a pulmonary infection appeared to be the precipitating cause. The occurrence of haemolysis did not presuppose that the patient had previously been subjected to any form of antileukaemic treatment and evidently was unrelated to the severity of leukocytosis, the size of the lymphomas, or the degree of splenomegaly. True, the spleen was very large in a few cases (Nos. 3, 4, 12) but in 6 (cases 2, 6, 7, 9, 10 and 13) it was impalpable or extended only a couple of cm below the costal border. A so-called "aplastic" haemolytic crisis was not observed in the present series, and obviously not either in other series of lymphatic leukaemia reported in the literature. Fig. 1 shows that the occurrence of a haemolytic complication is of no prognostic significance to the leukaemia.

Treatment

The result of splenectomy performed in 5 instances (cases 3, 4, 5, 8, and 13) may be seen in part from fig. 2.

In case 3 the result was striking and lasted for years. The spleen weighed almost 3 kg. There were signs of severe immunization, and microscopic examination of the spleen, which contained numerous reticulum and plasma cells,

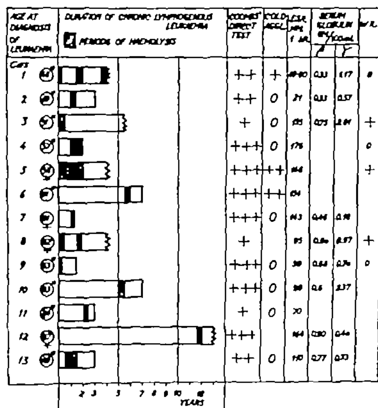


Fig 1 Survey of haemolytic periods during the course of chronic lymphatic leukaemia in 13 cases. Also, some laboratory data (cf. the case histories of cases 1—13).

The immunization manifested itself in a positive usually strongly positive Coombs direct test, although in 3 instances it was only weakly though indubitably positive. At least 3 cases also had an elevated cold agglutinin titre, but in no case was there a question of the cold agglutination syndrome. In case 5 the presence of the cold agglutinins evidently meant that transfusion reactions occurred when the transfused blood was below body temperature, while blood at 37° C did not cause any reaction Each time the Coombs test was positive all the patients but one showed signs of hyper haemolysis. On the other hand, Coombs test was negative in several periods despite unmistakable signs of haemolysis.

The erythrocyte sedimentation rate (E S R.) which was low in uncomplicated cases of chronic lymphatic leucaemia, was generally greatly elevated during periods of haemolysis (cf fig 1) and returned to normal when the haemolytic phase was over Not infrequently patients with chronic lymphatic leukaemia have immune paresis, especially a low γ-globulin (19 32) This may occur also in patients with A. I H. A., e g our cases 2 7 9 and 12 with hypogammaglobulinaemia. In case 7 the value was particularly low (0 18 g/100 ml) and in cases 1 and 7 there was a low concentration of β-globulin. In case 3 the electrophoretic diagram was unusual in showing a large, wide γ peak representing no less than 2.61 g/100 ml And indeed, this case was characterized by a feature unusual in chronic lymphatic leukaemia viz. proliferation of plasma cells in the bone marrow and spleen. In 3 cases the W R was what is called false positive.

The anaemia was generally severe, of rather sudden onset, almost in the form of a haemolytic crisis, and in that event values of 3—5 g/100 ml Hb. were not

by a few transfusions. As soon as re mission had been obtained an attempt was made to reduce the dose of steroid, and in some cases the treatment could be discontinued within a few weeks, while in others it had to be continued for months. The initial dosage was 30—60 mg daily In a number of cases, corti costeroid was the only treatment, while in a few it was combined with splenectomy (case 13 phase 2) and X-ray irradiation to the spleen or lymphomas.

Case 1 A 48-year-old man admitted with febrile jaundice. Hb. 4.5 g/100 ml, reticulocytes 3—4 %, leukocytes 40,000 98 lymphocytes. The spleen was 8—9 cm below the costal border and he had moderate lymphomas. E. S. R. 49 mm. Despite prednisone, 30 mg daily and X-radiation to the spleen whereupon the W. B. C. returned to normal, the Hb. went on falling. H had, therefore, 5 transfusions of 1/2 l blood, but it was not until the X-ray therapy had been finished, the spleen diminished to an almost normal size and the prednisone increased to 40 mg daily that the haemolysis could be controlled. Thus, intensiv treatment had been continued for 2 months. Thereupon, Coombs' test was negativ and the E. S. R. normal Hb 12—14 g/100 ml for 2 years, but in the course of pneumonia caused by M albicans new haemolytic phase occurred with jaundice, Hb. 5.8 g/100 ml, and an E. S. R. of 90 mm The liver was impalpable and lymphomas moderate. Coombs direct test was now again positiv and remained positiv during the next 4 months. During treatment, this time with prednisone alone, for total of 10 days and with an initial dose of 60 mg, the E. S. R. returned to normal and the Coombs direct test turned negative The patient has now been followed up for 4 years. The Hb level has remained 13—14 g/100 ml and the E. S. R normal, but during the past year Coombs test has again become positive However the T1/2 Cr⁵¹ was now completely normal (26 days

Case 2 1 49-year-old man generalized lymphomas were found by chance. They proved to be llak an aleukaemic lymphatic leukaemia. E. S R 4 mm, γ-globulin only 0 45 g/100 ml, Hb. 14.6 g/100 ml. Six months later X-ray irradiation to the mediastinum, since the mediastinal lymphomas ga rise to cough. Five months later moderat naemia, Hb. 11.0 g/100 ml, E. S. R. 21 mm, and Coombs direct test moderately positive retic ulocytes 0.5—0.8 serum bilirubin 0.5 mg/100 ml, but in th lymphatic bone marrow there were 7 % normoblasts, and the serum iron was 0 154 mg/100 ml. No splenomegaly Two transfusions resulted in only short lasting increase in haemoglobin, but the E. S R. dropped in the course of a month to 4 mm and the Coombs direct test remained negativ for 9 months and 18 months respectively

Case 3. A 51-year-old man with pneumonia was found to have spleno-hepatomegaly Hb. 8.3 g/100 ml W B. C. 4,280, 90 lymphocytes, sternal marrow 90 lymphocytes, 8 plasma cells. E. S. R. 150 mm, platelets 18,650 Aspiration biopsy from the spleen showed 10 reticulum and plasma cells, 90 lymphocytes. Thelius electrophoresis revealed wide γ peak of 2.61 g 100 ml, W R false positive reticulocytes up to 4.0 Coombs' direct test weakly positive, — leukocyte agglutinin. The spleen was removed. It weighed 2,800 g, and microscopic exami nation showed that it contained number of reticulum and plasma cells as well as well-preserved lymphatic nodules. Myelomatosis and macroglobulinaemia could be ruled out by Tiselius electrophoresis (Dr N Harboe) and ultracentrifugation (Dr R. Djurtoft) After the operation, the haemolysis ceased and Coombs test became negative. Four years later the patient was still working, with Hb. level of 12—11 g/100 ml, W B C. 10,000—20 000, 92—99 of which were lymphocytes.

Case 4 A 57-year-old man w th hronic lymphatic leukaemia of one year duration (generalized lymphomas, large spleen, W. B. C. 160,000, E. S. R 5—7 mm) developed anaemia within few weeks, the Hb. being 5 g/100 ml, reticulocytes 4 0 E. S. R. 176 mm, Coombs direct test moderately positive. A diffusely leukaemic spleen of 2,200 g was removed. Thereafter the E. S. R. was normal and so was the reticulocyte count, but the Hb.

indicated that splenectomy had eliminated a large part of the antibody producing apparatus and thus the spleen had been an important factor in the auto-immunization process. For the next 4 years no treatment was required and today the patient is working full time. The Hb level has been satisfactory ever since except at one time, when the patient had a large retroperitoneal abscess.

In case 4 likewise splenectomy resulted in a pronounced, but short lasting decrease in haemolytic activity. Later it was found that apparently the same result could be obtained by cortisone. In this case too the spleen was very large, weighing more than 2 kg but the microscopic appearance was dominated by lymphocytes.

Case 5 had more moderate splenomegaly. This patient also had laboratory findings indicating hyperimmunization and the spleen contained numerous reticulum cells. However the result of splenectomy was poorer than that of 4 weeks cortisone therapy given about 1 year later and evidently resulting in satisfactory stabilization of not less than 18 months duration.

Patient No. 8 was splenectomized initially. The spleen was moderately enlarged. Reticulosis was not striking, and the effect was fairly short lasting. Later during permanent cortisone medication a stable period of 2 years was obtained.

In one of the cases (No. 13) X radiation of the enlarged spleen was tried first, as the patient was 89 years of age and had bundle branch block. However the effect of the irradiation was maintained for only 2 months. Then a new crisis occurred, and splenectomy was performed. For a few weeks before and after the operation prednisone was administered. The result was entirely satisfactory.

This spleen too was a large one (480 g) and microscopic examination revealed a number of reticulum and plasma cells.

In other words, the result of splenectomy appeared to be good in 2 cases (3 and 13) whose spleens contained a striking number of plasma and reticulum cells and weighed 2,900 and 480 g respectively. A moderately good result was observed in case 5 whose spleen was of medium size and showed some reticulum-cell hyperplasia on microscopic examination. A poor result of splenectomy was found in cases 4 and 8. Case 4 had a very large spleen without an appreciable amount of reticulum cells or plasma cells. Case 8 had a medium-sized spleen also without hyperplasia of the reticulo-endothelial system. Case 13 also had short lasting pre and postoperative prednisone therapy which may have had a large share in the good therapeutic result. At any rate, it was observed in two instances (cases 1 and 5) that cortisone — as the only treatment and administered for only a few weeks — entailed improvement sustained for a long time.

While several workers state that splenectomy should be reserved for selected patients, the criteria of selecting the patients do not appear to be quite clear. From our series, though very small indeed, one might be tempted to conclude that the result of splenectomy would be expected to be good if the spleen is distinctly enlarged and contains numerous plasma and reticulum cells. A preoperative aspiration biopsy may afford this information (cases 3 and 8).

Like other authors (28) we have used corticoid medication as the main method, especially prednisone. In several instances this proved sufficient treatment, supplemented in some particularly severe cases

Coombs' direct test positive. In addition, cirrhosis of the liver and slight splenomegaly On prednisone continued for total of 2 1/2 months, initial dose 30 mg daily the condition underwent an enormous improvement and the E. S. R. returned to normal. The Hb. remained between 13 and 14 g/100 ml during the subsequent year

Case 11. A 64-year-old man, admitted in 1954 with herpes zoster was found to have generalized lymphomas, W B. C. 5,600 with 92 % lymphocytes. Treated with large doses of X-rays to multiple sites. He had 7 attacks of pneumonia during this period and therefore had several injections of human γ-globulin, although the γ-globulin in the serum was 1.3 g/100 ml. E. S R. bout 20 mm, Hb. 12—14 g/100 ml through 18 months. After the disease had persisted for 2 years, the Hb. went down to 10 g/100 ml during an attack of pneumonia. The E. S. R. was 70 mm, and Coombs' direct test eakly positive. Owing to general symptoms, he required X-ray therapy all the time. Died 6 months later in febrile cachexia. Shortly before his death, Coombs test had been negative.

Case 12. I 67-year-old woman lymphomas and lymphatic leukaemic blood were found by chance in 1946. She was treated for short time with urethane Followed up t brief intervals ever since. Hb. 10.5—14 g/100 ml. Thirteen years later however the Hb. rather suddenly dropped to 5.0 g/100 ml and the serum bilirubin to 1.6 mg/100 ml. The reticulocytes were 10—15 %. Coombs direct test as strongly positive, and the E. S. R. 164 mm. The spleen was about 10 cm below the costal margin. Only moderate lymphomas. Three portions of blood were administered and prednisone, 60 mg daily for 7 days, and thereafter in slowly decreasing doses. Within the first eek the E. S. R. fell to 9 mm, and within two weeks the reticulocyte count had returned to normal. Sixteen days after admission, the spleen was no longer palpable, the Hb. was rapidly rising, but the Coombs test did not turn negative until year had lapsed. Six months after the haemolytic phase prednisone could be discontinued, and the condition has since remained satisfactory for year

Case 13. An 80-year-old man was admitted with a Hb. level of 7 6 g/100 ml, serum bilirubin 2.9 mg/100 ml, reticulocytes 4 4—7 %, E. S. R. 110 mm, Coombs direct test moderately positive, W B. C. 36,000 with 84 lymphocytes. The spleen was slightly enlarged. Owing to heart failure with bundle-branch block, the patient was not given prednisone but instead blood transfusion and X-rays to the spleen, but the haemolytic activity appeared to be unchanged. Three months after the first admission he had another haemolytic crisis (Hb. 6 g/100 ml, reticulocytes 45—50 %) serum bilirubin 2.4 mg/100 ml, E. S. R 130 mm, Coombs direct test still positive. Prednisone, 40 mg daily was now given, and 6 blood transfusions. The spleen (480 g) was removed (microscopic examination showed number of reticulum and plasma cells) and within 3 months prednisone could be withdrawn. During the past 2 1/2 years the patient has been well and there has been no crises.

Discussion

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Case 5 A 58-year-old woman was admitted with a Hb of 5 0 g/100 ml, reticulocytes 6 serum bilirubin 1 1 mg/100 ml, E. S R. 146 mm, W R. false positive W B. C. 2 000 10 *lymphocytes, Coombs direct test moder*ately positive. A cold agglutinin was found in the serum. After 8 portions of blood which gave rise to severe *febrile* reactions unless the blood was 37° C when infused, a rather large spleen was removed (5 × 15 × 22 cm) Microscopic examination showed considerable reticulosis. The haemolysis persisted and the patient had subclinical jaundice with reticulocytes of up *to 10* E. S. R about *100* mm and the Coombs test was still positive. Eighteen months later she had a haemolytic crisis, 8 g/100 ml Hb- reticulocytes 30 % serum bilirubin 3.0 mg/100 ml, 20 % erythroblasts *in the blood ACTH for 5 days and* cortisone for 3 weeks had a prompt effect, so that a blood transfusion could be avoided *The E. S R dropped perceptibly and here*after the haemolytic tendency was only slightly marked, the Hb. level keeping fairly constantly at *10—11 g/100 ml through* approx. *2 years.* Then, the patient succumbed to her leukaemia combined with enormous hepatomegaly and leukaemic infiltration of the brain

*Case 6. A 61 year-old man with lympho*sarcoma. Five years later (in 1953) he developed lymphatic leukaemia with a W B C of 17 000 with 70 % lymphocytes. Hb. 10 3 g/100 ml, normal reticulocyte count, E. S R. 143 mm, but a strong cold agglutinin One year later the condition was unchanged, but the Coombs test was negative. Died in his home shortly after

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Case 8. A *62* year-old woman with splenomegaly Hb. 9.3 g/100 ml, Coombs direct test weakly positive E. S. R. 35 mm, reticulocytes *2 6* 30 % of the bone marrow cells normoblasts. Aspiration biopsy of the spleen contained 97 lymphocytes, other wise myeloid cells. W B. C. 3 000 with 81 % lymphocytes. After splenectomy (measurements 15 × 18 × 10 cm) the Hb. rose, but *only for a few weeks, and the Coombs test* at once became negative Nine months later the E. S. R. was 103 mm, Hb. 8.6 g/100 ml, reticulocytes 4.2 Coombs test was again positive, W B. C. 104 000, with 98 % lymphocytes. *On prednisone 15 mg three times daily* and TEM, Coombs test soon turned negative and the E. S R returned to normal. Prednisone *therapy was continued through more* than 2 years, and the Hb. remained at about 12 g/100 ml. The general condition was rather good.

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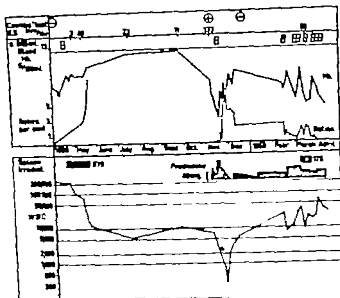


Fig. 3. During year follow-up on case 15 with chronic myelogenous leukaemia, variations in the E. S. R., Hb. reticulocyte and white cell counts are demonstrated, first in connection with X-ray irradiation of the spleen. Six months later in November 1958, the patient had an aplastic crisis for which successful prednisone therapy was instituted.

been 11 two months previously was now 133 mm, and Coombs direct test, which had been negative at the time of the first admission, was now weakly but unmistakably positive. Serum bilirubin 0.4 mg/100 ml, W. R. false negative, immune globulins normal. The spleen was enlarged, down to the iliac crest. On prednisone, as shown in fig. 3, the W. B. C. rose, and 7 days after the institution of the treatment the reticulocyte count rose to 6—7 % and the Hb. rapidly increased far more than could be accounted for by the two portions of blood. During the treatment the spleen rapidly decreased in size. At the time of admission it had been tender and enlarged, reaching to the midline, while at the end of 8 days it was impalpable. Six weeks later Coombs' test had become negative. Three months later the patient had blast crisis and died.

The latter patient is of particular interest, because in her case the auto-immune haemolytic anaemia was aplastic. This crisis ceased after one week's treatment with prednisone, after having lasted for at least a fortnight. This is one of only two "aplastic" crises among quite a large number of haemolytic phases observed by the author n 41 patients with A. I. H. A. Crises of this nature appear to have been encountered on ery rare occasions (2, 23) in cases of A. I. H. A., but they have been reported *to* be *not* infrequent in spherocytosis and to be seen in a few cases of sickle-cell anaemia. The duration of these crises has ranged from 7 to 14 days. While the platelet count was approximately normal, the acute "aplasia" in our case affected also the granulopoiesis. This may also pply to the aplastic crisis in congenital spherocytosis.

In stem-cell leukaemia A. I. H. A. appears to be a very rare complication although increased red cell destruction is common (1 a). In the literature, the author could find only one case in which the Coombs test was positive, and this was an atypical case in which the leukaemia was congenital (27). Osgood found haemolytic anaemia in 1 out of 48 patients (24) while the present cases (14 and 15) occurred among 27 consecutive cases of chronic myelogenous leukaemia.

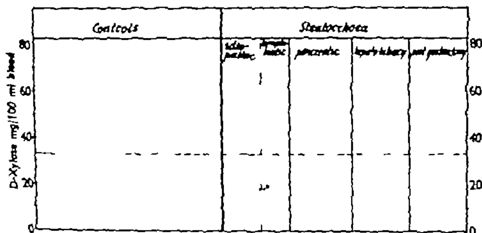


Fig. 2. The maximal concentration of D-xylose in blood.
— untreated patients. — patients on gluten-free diet. — patients after drainage of ascites. Symbols in perpendicular alignment belong to the same patient.

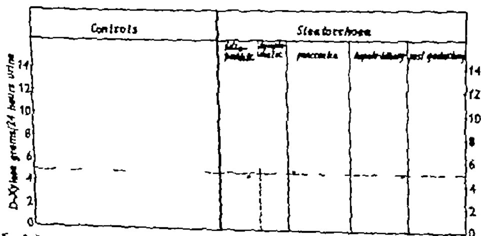


Fig 3 Excretion of D-xylose in 24 hours urine. Legends see fig. 2.

was usually reached 1.5 hours after administration of the xylose, but this could occur after 1 2 or occasionally 3 hours. In patients with absorptive dysfunction the values were definitely lower and the maximum value was reached later (fig. 1 bottom curve and circles).

The results of the xylose test were expressed as the maximum concentration of D-xylose in blood and the excretion of D-xylose in the 24 hours urine. In the control group the maximum concentration of D-xylose in blood (mg/100 ml) varied within the limits 38 and 74 with a mean of 50 and a standard deviation of 8.25. Consequently values within 50 ± 16.5 (= 33.5—66.5) can be expected in 95 per cent of subjects without gastro-intestinal disease. The urinary excretion of D-xylose (g/24 hours) varied

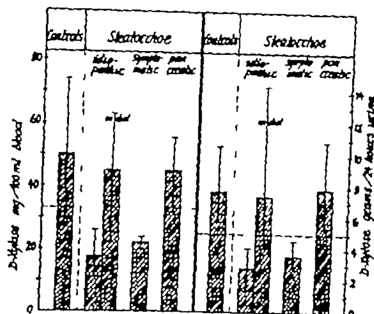


Fig 4 D-xylose maximal concentration in blood, and urinary excretion in 24 hours urine. Top of column indicates mean values. Thin line indicates limit values.

within the limits 5 8—10 6 with a mean of 7 7 and a standard deviation of 1 35 Consequently values within 7 7 ± 2.7 (= 5 0—10 4) can be expected in 95 per cent of subjects without gastrointestinal disease.

It is apparent from table II and figs. 2 3 and 4 that patients with absorptive dysfunction had significantly lower maximal blood xylose concentration and urinary xylose excretion than the controls.

In 6 patients with idiopathic steatorrhoea the maximal xylose concentration in blood varied within the limits 11—26 mg/100 ml, while the excretion varied within the limits 1 4—4 1 g/24 hours. In 4 patients with symptomatic (enterogenic) steatorrhoea the corresponding values were 20—24 mg/100 ml and 2.3—4 6 g/24 hours respectively

On a gluten-free diet the results of the xylose test improved in the 6 patients with idiopathic steatorrhoea, becoming completely normal in 5 of them (figs. 2 3 and 4) An unquestionable rise in the values obtained with the xylose test was observed on the 4th to 6th day of dietary treatment, while abnormally low values did not reappear until 2 to 3 weeks after discontinuation of the diet. Within the time of observation (1 to 4 years) continued treatment resulted in a significant reduction of the steatorrhoea in all patients. In only one patient did the steatorrhoea disappear completely

Among 10 patients with severe malabsorption due to pancreatitis or pancreatic cancer the xylose test gave normal values in 9 (table III and figs. 2 3 and 4) In one patient — an excessively emaciated woman with pancreatic cancer — the maximal blood xylose concentration was slightly abnormal whereas the urinary excretion of xylose was just normal.

In figs. 2 and 3 9 patients are recorded under the heading hepato-biliary steatorrhoea. The first 5 patients had pronounced jaundice due to acute hepatitis or biliary obstruction. In these cases the xylose test gave normal results and the same applied to 2 cases of cirrhosis without demonstrable ascites. The last 2 cases in this group were patients with immense ascites. In both patients the xylose test gradually im-

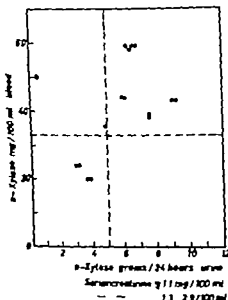


Fig. 5. Relationship between maximal blood xylose concentration and excretion of D-xylose in 24 hours' urine as found in patients with normal and decreased renal function.

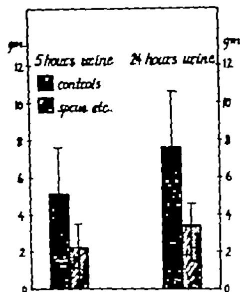


Fig. 6. Excretion of D-xylose in 5 hours and 24 hours urine.

proved as the ascites was drained (represented by crosses in the figures)

Finally table IV and figs. 2 and 3 refer to 8 patients with manifest steatorrhoea after partial gastrectomy (Billroth II and Polya). The results of the xylose test were normal in all cases except one where a jejunal biopsy specimen had shown some atrophy and inflammatory changes.

In all patients mentioned above the plasma creatinine concentration was below 1.1 mg per 100 ml, indicating a normal renal function. In these patients — as shown in the diagram (fig 5) — the correlation between the maximal blood xylose concentration and the 24 hours urinary xylose excretion was subject to some scattering. Nevertheless either can be used without significantly altering the outcome of the test. In addition fig 5 demonstrates that renal insufficiency is accompanied by an essential reduction of the xylose excretion.

From fig 6 it is evident that it is only possible to get well defined results with no overlapping between normal and pathological cases as long as the xylose determinations are carried out on the 24 hours urine.

Discussion

Experience with the D-xylose test is still rather limited. However all published reports as well as the present material have confirmed the value of this test in the evaluation of intestinal absorption.

The xylose test appears to provide reliable and rapid information concerning the effect of treatment in patients with idiopathic steatorrhoea. On the other

hand, after discontinuation of an effective therapy it may take several weeks before the results of the test become pathological again Accordingly a diagnostic xylose test should always be performed before any treatment is instituted.

Although the xylose test is well adapted for the examination of cases with idiopathic and symptomatic intestinal absorptive disturbances, there is no real connection between the results of the test and the degree of steatorrhoea. It has further been shown that even if the xylose test becomes normal in successfully treated cases of idiopathic steator rhoea some degree of steatorrhoea will still persist in most instances. Thus it must be kept in mind that the xylose test may easily fail in mild steatorrhoea.

In patients with steatorrhoea due to digestive disturbances the results of the test were normal with a single exception an extremely emaciated woman with pancreatic cancer In this and in other similar cases a compromised absorption may be due to secondary intestinal damage following malnutrition.

In post-gastrectomy patients with steatorrhoea the results of the xylose test were usually normal In only one patient with a microscopically verified jejunitis this was not the case. (In a large group of partially gastric resected patients examined after conclusion of the present work the results of the test have been without exception normal.)

It is often difficult to determine whether a steatorrhoea is due to defective absorption or digestion. In this field the xylose test probably represents a notable ad vance. Thus a pathological issue of the test will indicate an absorptive defect while a normal result in a patient with steatorrhoea will indicate a digestive insufficiency

According to previous studies (1 6) the urinary xylose excretion furnishes the most reliable measure of intestinal absorption. In the present material the blood xylose concentration appears to be equally well adapted to this purpose. Although xylose is mainly excreted during the first 5 hours after administration of the usual dose, it is recommended — contrary to suggestions by earlier authors — to determine the 24 hours urinary xylose excretion. This discrepancy of opinion is probably due to the fact that many of the patients in the present material are old and consequently have more or less incomplete voiding As the quantitative collection of urine may be unreliable it is advisable to combine determinations on blood and urine. This procedure affords a mutual control as well.

In patients with reduced renal function the urinary excretion of xylose is useless as a measure of intestinal absorption. Further it is still unsettled whether the blood xylose values may be of use in these cases.

In 6 diabetics investigated, of whom 4 were insulin-treated, the results did not deviate essentially from those ex pected

Apart from slight and transient diar rhoea in one control patient, the test did not give rise to any complications even in seriously ill patients.

Summary

The D-xylose absorption tolerance test consists of oral ingestion of 25 g of D-xylose followed by determination of the blood concentration and the urinary excretion of this substance

The evaluation of the test was based on the maximum concentration obtained

in blood (after 1 2 or 3 hours) and on the urinary excretion during the first 24 hours.

Thirty-three controls and 57 patients with various malabsorption syndromes were studied.

I the control group the mean values ± 2 standard deviations were 50 ± 16.5 (33.5—66.5) mg/100 ml blood and 7.7 ± 2.1 (5.0—10.4) g in the 24 hours urine.

In 6 patients with idiopathic steatorrhoea mean values and the limits were 17 and 11—26 mg/100 ml blood and 2.4 and 1.4—4.1 g/24 hours urine.

In 4 patients with symptomatic (enterogenic) steatorrhoea the mean values and the limits were 22 and 20—24 mg/100 ml blood and 3.6 and 2.3—4.6 g/24 hours urine.

Normal values were obtained in 9 out of 10 patients with pancreatic steatorrhoea (1 patient about lower normal limit) in 6 out of 7 patients with a partial gastrectomy and steatorrhoea, and in 7 patients with pronounced jaundice due to hepatic insufficiency or biliary obstruction. In 2 patients the results of the test became normal after drainage of immense ascites.

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Infectious Mononucleosis

An Epidemiological and Clinical Study

By

S. Belfrage

Infectious mononucleosis is probably a virus disease, of which Tidy (1934) distinguished three types according to the clinical picture, namely lymphonodular anginose and febrile, a disease with a peculiar blood picture dominated by mononuclear cells (Sprunt & Evans 1920 Tidy & Morley 1921) and typical serological reactions. In 1932 Paul & Bunnell demonstrated the presence of the heterophil antibodies in this disease. Paul-Bunnell's reaction has been made more specific by the establishment of an absorption pattern by Davidsohn. Many investigators believe that P. B. positive and P. B. negative cases of mononucleosis represent different clinical entities (Hoagland, Shubert, Hobson, Eyquem).

The incidence of mononucleosis was described by Hobson et al. (1958) on the basis of field studies in the district of Oxford. In that investigation all suspected cases were examined (P.-B. reaction and white blood cell picture). About 250 P.-B. positive cases were found, most of them of the anginose type with an incidence peak in the 20—29 year group and a seasonal increase in April—July. Almost all of the cases were sporadic, and the disease was equally common in both sexes. They also found 100 cases of P. B. negative mononucleosis, in about half of which tonsillitis was missing, with maximal incidence in boys and in the 0—9 year group. These 100 cases were equally distributed over the entire year, some of them occurring in small epidemics with an incubation time of about 1 week. Only 9 of them were admitted to hospital against 70 of the P. B. positive.

As mentioned, Hobson believes that P. B. positive and P.-B. negative "glandular fever" are of different aetiology. He bases his opinion on the above-mentioned differences in the seasonal, age and sex incidence. On the other hand, he found no marked difference between the clinical pictures of the two groups, though the signs and symptoms were generally less severe and recovery more speedy in the cases in which the P.-B. reaction was negative.

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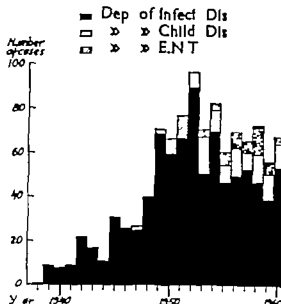


Fig 1 Annual number of cases of mononucleosis infectiosa cared for in Malmö 1938—1960

It might be questioned whether these differences in P B negative and P B positive cases of mononucleosis generally occur and, if so whether the difference is sufficient to regard the groups as representing two different diseases

In addition an infectious disease invariably giving a positive serologic reaction is surely something unique. Thus only about 50 % of all cases of primary atypical pneumonia are found to have cold agglutinins in their convalescent sera (Rivers & Horsfall 1959)

Material

In the present investigation — in contrast to Hobson's field studies, but like Thomsen's investigation — a hospital series of infectious mononucleosis *in the broad sense* of the word was investigated including P B. positive and P B. negative cases.

Altogether 424 cases of P B. positive and P B. negative mononucleosis cared for at Malmö General Hospital during the years 1954—1960 were studied. These cases represent all hospitalized cases of mononucleosis in a town with a population of somewhat more than 200 000 inhabitants during the 7 year period in question.

In some of the statistical studies (fig 4 tables I and II) the material was extended to include P B. negative cases *cared for in 1952—1953*

Diagnostic criteria

The most important criterion for the diagnosis of infectious mononucleosis was transient illness with *swelling of the lymph nodes and/or spleen* and with a blood picture dominated by mononuclear cells, many of McKinlay type. As a rule, at least 50 % mononuclear cells in the differential count and at least 15 % cells of McKinlay type were necessary for a case to be accepted as mononucleosis (Mason 1958)

The Paul-Bunnell test was performed in the vast majority of the cases with a clinical picture of mononucleosis. The reaction was read after 4 hours and after 18 hours in the cold, and in all positive cases the result was checked by absorption with guinea-pig kidney and, during the last 4 years of the investigation, also with ox-blood cells. A Paul Bunnell reaction positive in a dilution of at least 1/80 after absorption with guinea pig kidney was regarded as positive.

Most of the cases that satisfied the clinical and haematological criteria given above were P B. positive.

Of those cases in which the P B test was negative or not performed only those with absolute lymphocytosis with more than 4 000 mononuclear cells per mm were accepted. Almost all of the cases with a negative reaction without exudative tonsillitis were studied serologically for antibodies against toxoplasmosis, and 3 cases for antibodies against listeria monocytogenes. The tests excluded any such active infection.

Method

As a rule, the cases were followed with repeated blood cell studies and P B. tests. In addition, a large number of the patients were studied roentgenologically for any enlargement of the spleen and liver. Laboratory studies also included the thymol-turbidity test, determination of the serum aldolase, electrophoretic analysis of the serum proteins as well as assessment of the plasma fibrinogen. The results of the blood protein studies in these cases of mononucleosis will be the subject of a future paper

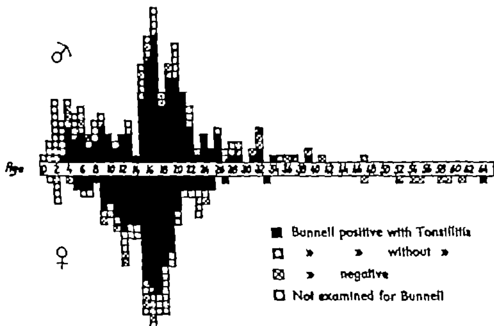


Fig. 2. Age and sex distribution of 424 cases cared for in Malmo 1934—1960.

Results

Before describing the findings in the series of 1934—1960 it might be convenient to refer to fig. 1 which gives the annual number of cases of mononucleosis admitted to various departments of Malmo General Hospital from 1938, when the disease began to receive wider attention. In this figure, however the cases of mononucleosis were not selected according to the above criteria. The increase in the number of cases until the beginning of the 1950 is due mainly to wider knowledge of the disease. During the last 7 years, i. the period covered by the present investigation, the incidence remained fairly steady with bout 60—70 cases per year

AGE AND SEX DISTRIBUTION

Fig shows the age and sex distribution of the 424 patients with P.-B. positive and P B. negative mononucleosis satisfying the criteria outlined above and treated during the years 1934—1960. Most of the cases were P B. positive, namely 343 as against 49 P B. negative. 32 patients, mainly young infants, were not studied for their reaction to the P B. test.

The figure shows a marked accumulation of cases in the late teen-ages. The median age of all patients was 16 years that of the P B. positive patients, 17 years. This peak in the teen-ages is due mainly to patients with a positive P B. reaction and with typical soreness of the throat with coated tonsils. The lower age groups — up to 9 years — included several cases with negative P B. reaction, particularly the lowest age groups. 7 infants 0—3 years of age had a negative reaction, while the youngest P.-B. positive patient was a 3-year-old child.

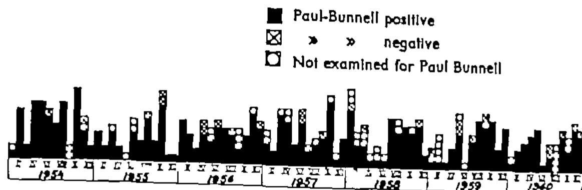


Fig 3 A. Time-distribution of 424 cases of mononucleosis cared for in Malmö 1954—1960.

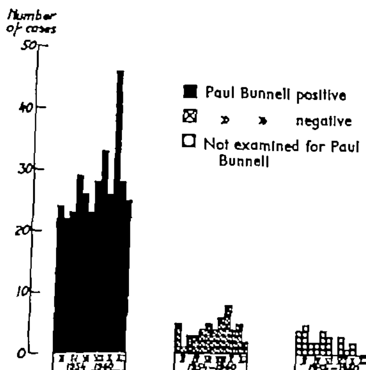


Fig 3 B. Time-distribution of the same cases (fig. 3 A) arranged according to time of year without distinction between years.

In the ages above 23 the relative number of P B negative cases increased, as did the relative number of cases without typical throat changes. Only about half of the cases were P B positive with typical throat changes. But there was one patient, a woman, with a positive P B reaction and exudative tonsillitis as old as 64 years

As to the sex distribution boys were more common among the children, especially in the lowest age groups, while girls were more common in the 9—14 year group In the ages around the incidence maximum the sex-distribution was roughly equal. From 20 years of age on, males were again predominant as far as P B positive mononucleosis is concerned, while many P B negative patients over 23 were females and all 8 patients over

50 were females. 49 % of the total material and 51 % of the P. B. positive patients were males.

Comments

A hospital series can never reflect the morbidity in the population if the disease is not very severe. In Hobson's series every third to fourth case of P. B. positive mononucleosis was admitted to hospital, as against only each eleventh P.-B. negative case. This would imply that the present hospital series contained a relatively higher percentage of the total number of P. B. positive cases than of the P. B. negative cases that had actually occurred in the population. Practically all of the P. B. negative infants in the present material had soreness of the throat, while about half of Hobson's P. B. negative group had no such changes. The throat changes were probably the main reason for admission of the P.-B. negative cases in Malmö. Children without throat changes, in other words cases corresponding to Pfeiffer's glandular fever, were probably not ill enough to need hospitalization. Only a few cases of this type are found in the present material.

All the ages are underrepresented in these hospital series but particularly children, because children generally have milder disease than adults — see later. Above all the P.-B. negative children are underrepresented.

All series published show an age-distribution with a very low frequency of cases above 30 years of age and an incidence peak in the lower age groups. Thomsen (1927—1938) and Bennike's (1949—1955) series from Copenhagen, which is situated fairly close to Malmö, agree almost completely with the present material, though the maximum incidence was somewhat later, namely in the 16—

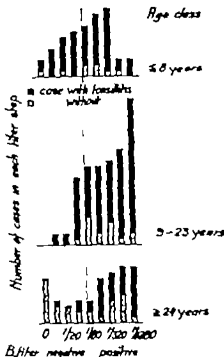


Fig. 4. Distribution of P.-B. titres in 223 cases of P.-B. positive and -negative mononucleosis, arranged in three age classes.

19 year group. This also applies to Leopold's German material from the 1930-ies. The sex distribution was also about the same as in the present material.

Other series show a much earlier maximum incidence such as Petride's Berlin-series of 1948—1950 or usually a later incidence maximum — 20—25 years — such as most American and British series (Press, Niederman, Hobson).

Seasonal variation

It is clear from the fig. 3 A and 3 B that no regular seasonal incidence could be observed in the P. B. positive or P. B. negative cases during the 7 year period covered by the investigation, but generally a slight increase was found in the autumn. Thomsen also found most cases during

the autumn months. Hobson on the other hand found an increased frequency of P B positive cases during the spring That investigation, however covered only 2 1/2 years.

COMPARISON BETWEEN P B POSITIVE AND P B NEGATIVE CASES IN THREE AGE CLASSES

Fig 4 compares the findings of P B negative and P B positive cases in three age classes. This diagram as the tables I and II is not based on exactly the same number of cases as that in the previous section The lowest and highest age classes have been increased by cases from the last few years before 1954 the middle age class is represented only by 96 personal cases from 1956—1959

The figure shows the cases in each age class grouped according to P B titre. In the two lowest classes the P B negative and positive cases together form a normal distribution of P B. titres, though the distribution for the 9—23 age group was at a higher level than that for the infants.

The highest age class in fig 4 on the other hand appears to show a heterogeneous distribution of P B titres. Of the P B. negative patients, many had no heterophil antibodies at all while the P B positive, like the positive teen agers showed a high median titre

The mean age in the two lowest classes of the P B. negative was 1—2 years lower than that of the P B positive, while in the highest class the mean age of the P B negative was much higher than that of the P B positive (table I) For the patients without soreness of the throat the mean age was 42 years in the P B. negative group and 30 1/2 in the P B positive.

The number of fever-days increased with every age class and in the two lowest classes it was slightly lower for the P B. negative than for the P B. positive, but in the highest class the P B. negative had fever for about one week longer than the positive.

In the two lower age classes most of the patients with P B positive tests and nearly all the P B negative had a sore throat. In the highest age class exudative tonsillitis was seen in 73 % of the P B. positive as against only 16 % of the P B. negative.

Nearly all of the patients in the two lower age groups and most of the P B. positive patients in the higher age groups had lymphadenopathy Of the patients without soreness of the throat in the highest age groups 64 % of the P B. positive but only 25 % of the P B. negative had palpable nodes.

Splenomegaly was seen in many patients in all age classes, but was somewhat less common among the children than among the adults — the former had not been roentgenologically examined as regularly as the adults.

Finally the white blood cell picture was roughly the same in all classes both for the P B. positive and the P B negative, thus about 10—13 000 W B. C. with 70—80 % mononuclear cells in the differential count

Table II shows the relation during childhood and adolescence between age on the one hand, and P B. titre duration of fever and sex distribution on the other hand In the P B. positive cases, but also to some extent in the few P B. negative cases the titre as well as the duration of fever increased and females become predominant with increasing age.

Comments

The P B. negative cases occurred particularly in the lowest and highest age

Table I. Relative frequencies and mean values of disorders in 223 cases of P.-B. positive and -negative mononucleosis arranged in three age classes

	Age class: ≦ 8 years, P.-B. reaction: Negative	≦ 8 years, Positive	9–23 years, Negative	9–23 years, Positive	≧ 24 years, Negative	≧ 24 years, Negative, ≧ 50 years	≧ 24 years, Positive	≧ 24 years, Cases without exudative tonsillitis: Negative	Cases without exudative tonsillitis: Positive
Number of cases	24	38	16	85	19	7	41	16	11
M. P.-B. titre	1/22	1/342	1/34	1/590	1/14	1/13	1/578	1/11	1/465
% males	88	63	38	58	53	0	76	50	91
M. age years	4.1	5.8	15.3	16.1	36.1	55.7	29.1	42.2	30.5
M. fever days	8.8	9.8	12.6	14.1	23.7	28.3	17.2	26.0	20.3
% throat +	92	84	94	83	16	14	73	0	0
% lymph nodes +	92	93	94	93	32	14	78	25	64
" spleen +	54	42	75	80	63	71	81	63	91
M. W.B.C. 1,000 cells/mm³	14.0	13.2	10.1	12.3	11.5	11.8	12.3	11.4	10.3
M. % mononuclear cells	72	72	70	77	77	81	80	77	80

Table II. Age in relation to P. B. titre, sex distribution and fever days during childhood and adolescence in mononucleous infections

	Age, years: 0–2	3–4	5–6	7–8	9–10	11–12	13–14
P. B. positive cases							
Number	–	9	16	13	21	20	22
M. P. B. titre	–	1/204	1/325	1/388	1/343	1/492	1/633
males	–	78	63	54	43	20	27
M. fever days	–	8.9	10.0	11.8	11.5	11.2	13.7
P. B. negative cases							
Number	6	10	4	4		5	
M. P.-B. titre	1/8	1/22	1/35	1/30		1/30	
males	83	80	100	100		0	
M. fever days	7.3	7.9	11.8	10.5		9.6	

lases. The P. B. negative children did not differ significantly from the P. B. positive but the P. B. negative patients above 23 years differed more markedly from corresponding P. B. positive cases.

THE BUNNELL NEGATIVE CHILDREN

The differences between P. B. positive and P.-B. negative children found — apart from the difference in P. B. titre — were a slight difference in age, in sex

distribution and duration of fever In the present material, however it was found that the P B, titre, sex distribution and duration of fever were to some extent dependent on the patients ages. In the P B negative group the average age was lower The lower age was probably the cause of the lower titre, the greater preponderance of males and the shorter duration of fever As to the P B titre, practically all of the P B negative infants had traces of antibodies and together with the positive cases they formed a normal distribution

Thomsen's large series from Copenhagen from years 1927—38 showed practically the same figures for the P B. titres and ages (see table in page 143 in Thomsen's book) Thomsen found that the average titre was lower in children 0—9 years old than in older children and relatively many were P B negative, particularly in the ages below 5 years. Only one of the 10 P B negative children below 10 years, a one-year old infant, had no antibodies at all. Similar findings were made by Bennike in a later Copenhagen series. In their material from the hospital for infectious diseases in Stockholm Vahlquist and co-workers, on the other hand, found that 14 children aged 5—9 years had roughly the same median titre as a large number of older children, namely 1/160—1/256 In the present material low titres were especially common among children below 6 years. It is possible that Vahlquist's series did not include so many children in this low age class. He did not give the individual ages of the children

Thus, in the present material the findings suggest, that the P.-B. positive and negative infantile cases of mononucleosis represented one and the same disease.

It should be stressed once more that nearly all of the P B. negative infants in the present series had typical throat changes. Half of Hobson's P B negative cases had no throat changes This author believes P B negative and P B positive mononucleosis to be different aetiological entities The discrepancy between the present findings and those of Hobson may be explained by difference in the composition of the materials. Vahlquist also recognizes as "pseudomononucleosis" a group of small children with negative P B reaction and lacking severe tonsillitis.

It is probable that the lowest positive titre for P B. in infants should be placed lower than 1/80 If the limit be taken as 1/40 — which Hobson seems to have taken — it would mean that about half of the P B negative children below 10 years like most of the P B. negative adolescents, would be transferred to the P B. positive group

THE P B NEGATIVE PATIENTS IN THE HIGHEST AGE CLASS

The highest age class of over 23 years (fig 4) appears to be heterogeneous. An admixture of other disease appears possible. As a matter of fact the titre distribution among the P B negative cases without throat changes in this class agrees fairly well with the distribution of the titres in a series of healthy blood donors — examined at the Institute of Bacteriology in Malmö — about half of whom had no antibodies at all and the remainder titres below 1/80

Also in Thomsen's material the distribution among patients above 24 years was irregular Of the 36 patients, 7 had a titre below the borderline (1/64) and 4 of these 7 had no antibodies at all.

The higher mean age the longer duration of fever the absence of demon-

strable throat changes as well as of enlargement of the lymph nodes among the P B. negative patients in this age class is compatible with the assumption that most of these had some other disease. As mentioned above, active toxoplasmosis was excluded serologically in these cases as was active listeriosis in 3 of the cases. One of the P.-B. negative patients, a 52 year-old woman, with a P B titre of 0 had 7 years previously had typical mononucleosis with a high P. B. titre.

The blood protein pattern in these P B. negative cases (to be published) showed largely the same abnormalities as in P B. positive mononucleosis. Thus, there was generally a slight increase of the alpha globulins but a marked increase of gamma globulins. The latter and thymol turbidity — likewise aldolase activity — was increased in most cases but somewhat lower than in the P B. positive cases. The reduction in the albumin was relatively greater than was usually seen in P B. positive mononucleosis. In the later course of some cases, anhaptoglobinaemia and a subnormal amount of fibrinogen were demonstrated, findings not seldom made in P B. positive mononucleosis (Nyman, Beifrage)

The P B. negative and positive cases in the highest age classes are, however as far as age is concerned, not strictly comparable because the difference in the median age was about 12 years. If those 7 P B. negative cases are selected which were above 50 years — only one P.-B. positive case was above this age — it will be found that all 7 were females, that the duration of fever as well as the reduction of serum albumin was somewhat greater but that the findings were otherwise essentially the same as in the entire group of P.-B. negative patients above 23 years (table I) Thus, the elderly patients in the P B negative group seems to be responsible for only part of the difference from the positive

Can the negative P B. reaction of these cases be due to incomplete heterophil antibodies?

In 4 cases belonging to this group of P B. negative patients the serum was studied for incomplete antibodies.

The P B. test was performed. The sheep blood cells were separated off, washed and then mixed with Coombs sera on the slide. In one case with a P B. titre of 1/20 agglutination was obtained with Coombs serum in a dilution 1/40—1/80 possibly suggesting the presence of incomplete antibodies. But the other 3 sera gave no agglutination. It is thus probable that in most of these cases the negative reaction was not due to incomplete antibodies.

Can it be a question of some other infection than P B. positive mononucleosis An infection that gives immunity is rarely particularly common in middle age. Such diseases can affect all ages but the maximum age incidence is usually earlier An infection with an incidence maximum among the elderly is *Zoster* As to Zoster it may be regarded as established that it is due to activation of a latent virus. Is it possible that the disease in these somewhat elderly individuals, frequently middle aged women may be explained by activation of a latent virus? The aetiology and pathogenesis in these P B negative cases is obscure.

Discussion

In the present material of P B. positive and P B. negative mononucleosis those patients in the age of maximal incidence showed the most uniform picture

with marked preponderance of P B positive cases of anginose type.

In children, especially young boys, a P B negative or rather a P B. weak mononucleosis occurred in association with typical throat changes and with a clinical picture compatible in all other respects with the P B positive mononucleosis in the same ages. No evidence was forthcoming to suggest that this P B negative mononucleosis in children was of a different aetiology than the genuine P B. positive mononucleosis — this is in disagreement with Hobson's opinion. On the other hand, the present series included hardly any P B negative children with lymphonodular mononucleosis. It is, above all, that group that is suspected of being of different aetiology

In higher age classes, *i. e.* above 23 years, in many *women in middle age*, an entirely P B negative mononucleosis was seen *with protracted fever but without exudative tonsillitis* and with inconspicuous *or no lymphonodular swelling*

This group of P B negative patients *with a relatively characteristic picture* appears to have passed unnoticed in the *literature. It has a certain similarity* with Tidy's conception of the febrile type of mononucleosis *before the time of the P B* reaction. The negative P B reaction is probably *not due* to incomplete heterophil antibodies. The possibility of an active *toxoplasmosis* was excluded Whether the group represents another disease or some special type of the same aetiology as mononucleosis is uncertain

Epidemiological features

The remarkable age distribution of mononucleosis was shown in fig 2 It somewhat resembles that seen in poliomyelitis in modern populations before the introduction of vaccination. The latter distribution is believed to be due to widespread infection of children and young adults, but with a greater resistance and a lower morbidity rate among children than among adults and with a general immunity over 40 years of age.

These remarks may also hold for mononucleosis. But poliomyelitis occurs in epidemics. *Mononucleosis occurs sporadically* all the year round. In the present *material of more than 400 cases there* were only 6 pairs of sibs. The infectiousness *of the disease must be low*

Many authors (Hoagland, Eyquem) *claim that it is*, above all, *an infection* spread by kissing This is possible, but it *is also possible that many small children* are infected — without a characteristical *clinical picture — during the first years* of life. This appears to hold above all for *the lowest social class*, whose members seem to be under-represented in this as well as in *Thomsen's series* of mononucleosis. This might help to explain the general immunity which appears to exist in the age classes above 30 years.

Summary

The material consists of 424 cases of infectious *mononucleosis representing* all cases treated within a 7 year period in hospital in Malmö, a town with more than 200 000 inhabitants.

Three hundred and forty three of the cases were P B. positive, 49 P B. negative and 32 were not studied in this respect. The youngest P B positive was 3 years old, the oldest 64 years. There was a marked incidence-maximum in the 16—17 year group In the highest and lowest age classes the number of P B negative cases was markedly high. No distinct seasonal variation was found for P B. positive or P B negative cases.

The 30 P B *negative infants* of whom practically all had exudative tonsillitis, did not differ from the positive cases except by a somewhat lower mean age, greater preponderance of males and shorter duration of fever As a rule, they had heterophil antibodies of low titre.

The lower mean age can probably explain all the other discrepancies. No evidence was found for any aetiology different from that in the P B. positive cases.

Of the 19 P B *negative cases above 25 years*, 16 had no typical throat changes. Their mean age was high, 42 years — 7 women were above 50 years. Most of the patients had no lymphonodular swelling and the fever was protracted. Half of the patients had no heterophil antibodies at all.

This group of somewhat elderly patients with P.-B. negative mononucleosis differed more distinctly from the P B. positive cases and might have been of some other aetiology

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Purulent Meningitis

A Review of 658 Cases

By

F. Quaade and K. P. Kristensen

Although the increasingly successful treatment of purulent meningitis is well documented in the literature, we have thought it worth while to review a large material from the last decade. Our motive is that the mortality especially from pneumococcal meningitis, is considerable in spite of the fact that the bacteria in question are always sensitive to most antibiotics and sulphonamides, and despite the increasing efficiency of our measures against complicating shock, fluid and electrolyte disturbances and respiratory insufficiency.

Material and results

Our material comprises all patients who have been treated for purulent meningitis in the Department of Communicable Diseases of the Blegdamshospital, Copenhagen, during the last eleven years (1949—1959) a total of 658 cases (table I).

We shall only briefly discuss the small group of patients whose meningitis had unusual etiology (table II).

Staphylococcus aureus, streptococci (including streptococcus fecalis) and E. coli (in infants) constitute the majority. The rest of the table illustrates the well known fact that a great variety of bacteria may occasionally cause a purulent meningitis. The high mortality is due to the fact that the group contains many septic and debilitated patients in whom meningitis was only one of many other diseases.

Our results for treatment of *meningococcal meningitis* (3.7 % mortality) are as good as in the most favorable reports from other countries. Our standard therapy is crystalline penicillin 300 000 units i.m. for adults and 150,000 units i.m. for children, combined with a large dose of sulphonamide. Autopsy findings for the ten deceased patients are shown in table X. We do not consider that there are grounds for altering our therapy.

An analysis of our group of 84 patients with *hemophilus meningitis* reveals that the eight deaths all occurred before 1955 and that all 36 cases admitted from 1955 to 1959 survived (table III). The mortality

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Table IV shows that our mortality from this type of meningitis was 17.3 % and that the number of fatal cases showed no tendency to decrease during the last eleven years.

In table V we have compared the 23 dead and the 110 surviving patients concerning some points that appear to be of prognostic relevance.

Ad 1. Confusion, somnolence and agitation were frequent in both groups. *Coma* — defined as deep unconsciousness with no reaction to even painful stimuli — was an unfavourable prognostic sign, as, with one exception, it occurred in all patients who died. The difference between the frequency of this sign in fatal and non-fatal cases is highly significant.

Ad 2. In accordance with previous reports pneumococcal meningitis was found to be especially dangerous for *elderly patients*.

Ad 3. Presumably some of the *convulsions* in the children were benign and caused by fever alone, but we found it impossible to distinguish reliably between febrile convulsions and the seizures that reflected cortical irritation from the meningitic process. The symptom occurred significantly more frequently among the fatal than among the surviving cases and was often followed by irreversible deterioration manifested by shock, stagnation of bronchial secretions and respiratory failure.

Ad 4. The increased frequency of *pneumonia* (diagnosed in vivo) hardly points to the lungs as the primary site of infection. In our opinion it reflects nothing more than the debilitated condition of our fatal cases and especially their deficient respiration. Six additional pneumonias were diagnosed post mortem.

Table IV. Purulent meningitis 1949—59. Pneumococci

Year	Number	Dead
1949	10	2
1950	11	2
1951	12	3
1952	11	3
1953	9	0
1954	11	1
1955	16	4
1956	10	1
1957	15	4
1958	10	1
1959	18	2
Total	133	23 (17.3%)

Table V. Pneumococcal meningitis 1949—59. Comparison between dead and surviving patients

Criterion	Dead (23)	Surviving (110)	t	Significance
1 Coma on admission	22	21	7.7	+
2 Age above 60	9	8	4.2	+
3 Convulsions	8	15	2.7	+
4 Pneumonia	8	14	2.6	+
5 Cell count below 1,000 mm³ on admission	12	28	2.5	+
6 Bacteremia	15	45	2.1	+
7 Below 25 mg% glucose in spinal fluid on admission	13	40	1.8	(+)
8 Pneumococcus types 1, 7 and 18	10	29	1.1	—

Table I Purulent meningitis 1949—59

Bacteria	Number	Total %	Male	Female	Dead	Mortality
1 Meningococci	267	40.5	155	112	10	3.7
2. Hemophilus (B. Pfeiffer)	84	12.8	46	38	8	9.5
3. Pneumococci	133	20.2	76	57	23	17.3
4 Coli, staph. aur listeria, and others	36	5.5	24	12	19	53 0
5. Unknown	138	21.0	86	52	15	10 9
Total	658	100 0	387	271	75	11 4

Table II Purulent meningitis 1949—59
Unusual etiology

Bacteria	Total	Dead
Staph. aureus	10	6
Streptococci	8	3
E. coli	6	4
L. monocytogenes	2	0
Proteus	2	2
Ps. pyocyanea	1	0
S. typhi murium	1	1
B. anitratum	1	1
Enterococci	2	1
Klebsiella	1	1
N catarrhalis	1	0
Gram diplococcus	1	0
	36	19 (53%)

Table III Purulent meningitis 1949—59.
Hemophilus (B Pfeiffer)

Year	Number	Dead
1949	4	0
1950	9	3
1951	11	2
1952	11	2
1953	7	0
1954	6	1
1955	3	0
1956	9	0
1957	7	0
1958	7	0
1959	8	0
Total	84	8 (9.5%)

1949—54 (8 of 48 = 16 7 %) is signifi cantly higher than that for 1955—59 (0 of 36) the t value being 2 55 We feel that this striking improvement of our results should be ascribed to the fact that before 1955 we did not replace penicillin with streptomycin until bacillus hemophilus had been demonstrated but in the later period treatment with streptomycin was commenced immediately in all cases of purulent meningitis that could not be classified at once. It goes without saying that our department will continue its present treatment of hemophilus meningitis, namely streptomycin varying with age and weight from 0 125 g × 2 to 1 g × 2 combined with serum and a large dose of sulphonamide. The reader is referred to table X for autopsy findings in the eight fatal cases.

Our treatment of *pneumococcal meningitis* comprises sulphonamides (formerly also specific serum from rabbits) plus crystalline penicillin The standard doses of the antibiotic was 300 000 units × 2 i.m. for adults and 150 000 units × 2 i.m for children but in some severely ill patients and in cases who did not respond satisfactorily the dose was raised to a maxi mum of 2 million units × 4

and 16 days. Autopsy was performed in 21 cases (table VII)

We were strongly impressed by the fact that our treatment was unable to prevent these gross manifestations of infection. They were present in all patients but one — the woman who died after 44 days in hospital.

The *purulent membranes* were several mm thick and covered the convexities of the brain like a cap occasionally they involved the base or followed the blood vessels into the brain substance. In this way continuity was established between hemispherical lesions and *brain abscesses* in three cases. One patient with non-purulent *fibrinous membranes* was remarkable, as his spinal fluid was opaque because of bacteria, but contained no leukocytes at all. In two cases there were extensive *emollitions* caused by both transversal sinuses being blocked by thrombi, and in one patient *cerebral incarceration* must have been the immediate cause of death. We have already mentioned that six of the 14 pneumonias had not been detected ante mortem. Finally we should add that two patients had *ulcerative endocarditis* with extensive destruction of the aortic valves.

There were 138 patients in whom we did not succeed in making a bacteriological diagnosis (table I) In the treatment of these unclassified cases it was the routine of the department to continue all three main therapeutics penicillin sulphonamide and streptomycin until there was clinical improvement, after which they were discontinued one at a time, beginning with streptomycin. Although it cannot be proved, we are inclined to believe that the group consists mainly of the types of purulent meningitis most commonly encountered in this country namely meningococcus, hemophilus and pneumococcus. Fifteen died, and autopsy was performed in 12. The duration of their disease and stay in hospital did not differ from the corresponding figures for our pneumococcal and hemophilus cases.

Table VIII Purulent meningitis 1949—59 Unknown etiology

Year	Number	Dead
1949	11	3
1950	12	1
1951	13	1
1952	9	1
1953	11	1
1954	14	0
1955	9	2
1956	8	0
1957	16	1
1958	21	3
1959	13	2
Total	138	15 (10.9%)

Table IX Purulent meningitis 1949—59 Treated before 1st lumbar puncture

I. Meningococci	78 of 267 = 29.2 %
II. Hemophilus (B. Pfeiffer)	37 of 84 = 44.0 %
III. Pneumococci	56 of 133 = 42.1 %
IV. Unknown	71 of 138 = 51.4 %

Table VIII shows that the number of deaths did not decrease during the eleven years. Especially there is no decrease after 1954 which might have been expected if the group consisted mainly of undiagnosed hemophilus cases. The comparatively high mortality (10.9 %) also makes it unlikely that the majority were undiagnosed cases of meningococcal meningitis, although we believe that there were a number of such patients. It seems justifiable to suppose that there is a con-

Table VI Pneumococcal meningitis 1949—59 Results of direct microscopy of spinal fluid from patients treated and untreated before admission

	Pos. microscopy	Neg microscopy	Total
With preliminary treatment	21	35	56
Without preliminary treatment	29	48	77
Total	50	83	133

Table VII Autopsy findings for 21 patients who died from pneumococcal meningitis

Purulent membranes	16
Fibrinous membranes	3
Thickened meninges	11
Pus in ventricles	7
Cerebral abscess	4
Sinus thrombosis	2
Incarceration	1
Pneumonia	14
Ulcerative endocarditis	2

Ad 5 It seems worth note that *low leukocyte count in the spinal fluid* proved to be a serious prognostic sign, and we may add that large amounts of pneumococci were often seen together with low cell counts. Agranulocytosis was not demonstrated in any of the patients on the contrary they showed varying degrees of leukocytosis in the peripheral blood.

Ad 6. The fact that a *positive blood culture* is correlated with mortality confirms previous reports.

Ad 7 A *lowered glucose content* in the spinal fluid occurred more often in the fatal than in the other cases, but the difference was not statistically significant.

Ad 8 We had the impression that infection with pneumococci of *types 1 7 and 18* had the gravest prognosis, but there was no difference between groups in this respect.

Contrary to our expectations, the average *duration of the untreated disease* was the same in the fatal and non-fatal cases 2.25 days for 12 fatal cases, and 2.33 days for 72 survivors. This must be explained by the fulminant course of the disease in many of the fatal cases.

Excessively high temperatures were seen with equal frequency in both groups.

Almost half the patients (56) had been *treated before admission* with varying (mostly insufficient) doses of antibiotics and/or sulphonamides. The frequency of this preliminary treatment — 9 out of 23 — among the fatal cases did not differ from the corresponding rate — 48 out of 110 — among the survivors.

There was probably an unknown number of patients with pneumococcal meningitis in whom preliminary treatment destroyed our chances of obtaining a bacteriological diagnosis. Such cases are to be found in the group of "unknown etiology" which will be discussed later However among our 133 patients in whom the diagnosis was verified by a positive culture, preliminary treatment did not hamper our possibilities of immediate diagnosis based upon direct microscopy of the spinal fluid on admission (table VI)

The time spent in hospital by the 23 patients who died from pneumococcal meningitis varied considerably one died immediately after admission, and 8 died within the first 24 hours one 70-year-old woman died after 44 days from intercurrent diseases after her meningitis had been cured. The survival-time for the others is evenly distributed between 2

and 16 days. Autopsy was performed in 21 cases (table VII)

We were strongly impressed by the fact that our treatment was unable to prevent these gross manifestations of infection. They were present in all patients but one — the woman who died after 44 days in hospital.

The *purulent membranes* were several mm thick and covered the convexities of the brain like a cap occasionally they involved the base or followed the blood vessels into the brain substance. In this way continuity was established between hemispherical lesions and *brain abscesses* in three cases. One patient with nonpurulent *fibrinous membranes* was remarkable as his spinal fluid was opaque because of bacteria, but contained no leukocytes at all. In two cases there were extensive *emollitions* caused by both transversal sinuses being blocked by thrombi, and in one patient *cerebral incarceration* must have been the immediate cause of death. We have already mentioned that six of the 14 pneumonias had not been detected ante mortem. Finally we should add that two patients had *ulcerative endocarditis* with extensive destruction of the aortic valves.

There were 158 patients in whom we did not succeed in making a bacteriological diagnosis (table I) In the treatment of these unclassified cases it was the routine of the department to continue ll three main therapeutics penicillin, sulphonamide and streptomycin until there was clinical improvement, after which they were discontinued one at a time beginning with streptomycin. Although it cannot be proved, we are inclined to believe that the group consists mainly of the types of purulent meningitis most commonly encountered in this country namely meningococcus, hemophilus and pneumococcus. Fifteen died, and autopsy was performed in 12. The duration of their disease and stay in hospital did not differ from the corresponding figures for our pneumococcal and hemophilus cases.

Table VIII Purulent meningitis 1949—59
Unknown etiology

Year	Number	Dead
1949	11	3
1950	12	1
1951	15	1
1952	9	1
1953	21	1
1954	14	0
1955	9	2
1956	9	0
1957	16	1
1958	21	3
1959	13	2
Total	158	15 (10.9%)

Table IX Purulent meningitis 1949—59
Treated before 1st lumbar puncture

I.	Meningococci	78 of 267 = 29.2 %
II	Hemophilus (B. Pfeiffer)	37 of 84 = 44.0 %
III.	Pneumococci	56 of 133 = 42.1 %
IV.	Unknown	71 of 158 = 51.4 %

Table VIII shows that the number of deaths did not decrease during the eleven years. Especially there is no decrease after 1954 which might have been expected if the group consisted mainly of undiagnosed hemophilus cases. The comparatively high mortality (10.9 %) also makes t unlikely that the majority were undiagnosed cases of meningococcal meningitis, although we believe that there were a number of such patients. It seems justifiable to suppose that there is a con-

Table X. Autopsy findings in 50 patients who died from purulent meningitis

Autopsy	Meningo-cocci (10)	Pneumo-cocci (21)	Hemophilus (B. Pfeiffer) (7)	Unknown (12)
Purulent membranes	2	16	7	6
Fibrinous membranes	0	1	0	0
Thickened meninges	0	11	5	1
Pus in ventricles	0	7	2	1
Cerebral abscess	0	4	0	4
Sinus thrombosis	0	2	0	0
Incarceration	0	1	1	0
Pneumonia	1	14	0	5
Ulcerative endocarditis	0	2	0	1
Suprarenal hemorrhage	2	0	0	0

siderable number of pneumococcal infections in the group Our impression is that the diagnostic difficulties increase when antibiotics and/or sulphonamides are given before admission the doses, though unable to cure the infection, modify the findings in the spinal fluid so much that an early and precise diagnosis becomes impossible. Absence of bacteria, abnormal morphology and staining of the bacteria low cell counts and predominance of lymphocytes jeopardize the etiological classification within the three main types of purulent meningitis and make it difficult to distinguish these from serous viral meningitis, tuberculous meningitis and meningitis caused by listeria monocytogenes. It therefore seems reasonable to check the frequency of preliminary treatment in our material of 138 unclassified cases, and to compare it with the three main groups of known etiology (table IX)

As we expected, the percentage of preliminary treatment was highest in group IV but it must be admitted that in almost half these patients the etiology remained obscure in spite of no such treatment having been given. The lowest incidence was found in the group of meningococcal meningitis. The great sensitivity of meningococci makes us inclined to believe that a number of patients who were in reality meningococcal cases are to be found among the patients given preliminary treatment in group IV

Table X shows a comparison of autopsy findings in the four main types of purulent meningitis.

Macroscopic signs of infection occurred in only two of the ten patients who died from meningococcal meningitis. This harmonizes well with the fact that these were fulminant cases of sepsis, they were all desperately ill on admission, and eight died from shock within 24 hours. The majority were young children of whom two proved to have suprarenal hemorrhage the autopsy findings for the rest were unspecific. In contradistinction to this, the three remaining groups have the following two points in common

1) The duration of the disease and of hospitalization was so long that treatment should have been able to be effective.

2) There is a high incidence of the signs of gross infection mentioned above

Discussion

As a considerable number of patients with purulent meningitis still die with gross infectious lesions of the brain and meninges, we feel that some improvement of the therapy is desirable. Concerning hemophilus meningitis it suffices to stress the vital importance of immediate administration of streptomycin. The cases of pneumococcal meningitis and purulent meningitis of unknown etiology have many traits in common. The efforts to reduce mortality in pneumococcal meningitis can be classified as follows.

Large doses of penicillin

By this means some reduction in mortality (from 62 to 38 %) was achieved already in 1949 by Dowling and his coworkers. A mortality in adult patients of about 25 % nevertheless seems to be the best we can hope for by intensifying penicillin treatment alone (Ribble and Braude 1958). The optimal dosage is still open to discussion, but most authors agree that it should considerably exceed the amounts usually given in pneumonia, as the characteristic fibrino-purulent membranes quickly relegate the antibiotic from vascular transport to pure diffusion.

Intrathecal penicillin

has been widely employed, especially at the beginning of the treatment but is being abandoned now as it leads to no convincing improvement in the results. It should be remembered that the inflamed meninges are permeable by penicillin, and that this antibiotic may cause arachnoiditis and block by local irritation. Single doses exceeding 15 000 units should be avoided.

Addition of other antibiotics

Chloromycetin, given alone (Singh 1957) or with penicillin and sulphonamide (Leblanc 1958) and tetracyclines (Rischbieth 1960) have not been proved to reduce mortality compared with materials efficiently treated with penicillin and sulphonamides. Lepper and Dowling (1951) even found antagonism between aureomycin and penicillin.

Steroids in combination with antibiotics and sulphonamides

This treatment has been employed as a short-term measure against infectious shock. Of more interest to our problem is its administration over a longer period perorally parenterally or intrathecally to combat fibrinous and purulent deposits (Ribble and Braude 1958, Leblanc 1958, Lasch 1960). The works cited deal with small materials or single cases but the results have been encouraging, especially those of Ribble and Braude, who had eleven survivors out of twelve patients. Intrathecal steroid is known to be without risk in purulent meningitis, but it can hardly be expected to influence fibrino-purulent membranes that already exist — and if these are not yet present, it seems more rational to let the steroid act via the blood. Of course, all steroid therapy should be started quickly in purulent meningitis, as it can only be hoped to prevent, and not to dissolve, the meningeal deposits.

Hibernation

The literature provides no systematic report proving the value of hypothermal treatment in pneumococcal meningitis, and our own material, in which terminal hyperthermia occurred in 5 of the 23

fatal cases allows of no opinion as to whether some might have been saved by hibernation therapy

Neurosurgical intervention

In a few cases trepanation with ventricular aspiration has been considered a life-saving measure against incarceration, ventricular empyema and concomitant cerebral edema (Rischbieth 1960)

Our own results and the reports in the literature have now led our department to introduce two alterations in its routine treatment of pneumococcal meningitis and purulent meningitis of unknown etiology

1) Penicillin dosage will be raised (300 000 units × 2 for infants—2 million units × 2 for adults)

2) *Half the patients, selected at random*, will be given steroids, beginning with a quick-acting preparation.

Summary

During the years 1949—59 a total of 658 patients have been treated in the Department of Communicable Diseases of the Blegdamshospital, Copenhagen for purulent meningitis (meningococci 40.5 %) hemophilus (B. Pfeiffer) 12.8 % pneumococci 20.2 % unusual etiology 5 5 % and unknown etiology 21 0 %) In meningococcal meningitis the mortality was 3 7 % the few patients who died were fulminant cases with irreversible infection shock, and their autopsy lesions were mostly unspecific. The total mortality in hemophilus meningitis was 9.5 %, but not one patient died after the introduction in 1955 of immediate streptomycin treatment. The mortality remained rather high in pneumococcal meningitis (17 3 %) and meningitis of unknown etiology (10.9 %) In pneumococcal meningitis the following signs indicate poor prognosis

1) Coma on admission.
2) Age above 60
3) Convulsions.
4) Pneumonia.
5) Low cell count in the spinal fluid on admission.
6) Bacteremia.
7) Low glucose content in the spinal fluid on admission.

Despite treatment being given for a reasonable length of time, autopsy revealed surprisingly gross lesions in the meninges and cerebrum of almost all fatal cases. In this respect, the group of unknown etiology closely resembles the group of pneumococcal infections, although we suppose it contains a number of patients with meningococcal meningitis in whom the bacteriological diagnosis was made impossible by treatment given before admission Mention is made of some possible ways to reduce mortality in pneumococcal meningitis and purulent meningitis of unknown etiology

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Small-vessel Disease of the Lower Extremity in Diabetes Mellitus

On the Pathogenesis of the Foot-lesions in Diabetics

By

Jørgen Pedersen[1] and Steen Olsen[2]

In an endeavour to learn more about the challenging problem of lesions of the diabetic's lower extremity an investigation was begun in 1959 at the Bispebjerg Hospital, including surgical (Dr K. Harry Sorensen) anaesthesiological (Dr W Dam) neurological (Dr E. Skinhøj) radiological (Drs. Bjerre and Fahrenkrug) as well as medical examination and treatment. While this study has been of value for the practical management of patients in the hospital, the results in main confirm the findings previously published from the Bispebjerg Hospital (J. Pedersen 1960).

As part of this investigation, however microscopical examination of biopsies of skin and muscles of the legs as well as of few amputated extremities of diabetics has been made according to the methods described recently by Goldenberg et al. (1959). After a short survey of their results we here describe our histological findings and present a concept of the pathogenesis of the lesions of the feet of diabetics.

The material of Goldenberg et al. (1959) consisted of 152 amputation specimens from the files of the hospital, varying in extent from mid thigh amputation to amputation of a single toe. 92 extremities came from diabetic patients and 60 from non-diabetic, one half with obliterative vascular disease. Large and small-vessels were studied primarily for evidence of endothelial proliferation, alterations of elastic structures and changes in polysaccharide-containing components. Successive sections were stained with hematoxylin-eosin by the Periodic Acid-Schiff (PAS) method and by the colloidal iron technique of Rinehart and Abul-Haj for mucopolysaccharide; and by the Verhoeff—van Gieson technique for the demonstration of alterations in elastic fibre, connective tissue and muscle. A section was subjected to microincineration.

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Table 1 Data for the 20 diabetics (12 males and 8 females)

		Range	Average
Age (years)	Males	15—81	58.3 } 57.5
	Females	39—78	56.5
Duration of diabetes	(years)	0—31	12.4
Sedimentation rate	(mm/1 h)	6—153	51.2
Haemoglobin	(g/100 ml)	9.4—15.0	13.1
Serum cholesterol	(mg/100 ml)	129—491	238.0 (18 cases)
Protein in spinal fluid	(mg/100 ml)	44—99	65.4 (10 cases)
Protein in serum	(g/100 ml)	5.1—8.9	6.95 (18 cases)

Insulin treatment 18 cases. Proteinuria 11 cases. Serum creatinine abnormal in 6 cases, max. value 3 mg/100 ml. Diabetic retinopathy 9 cases (none of the proliferative type).

In the small arteries and arterioles a characteristic lesion was found in 92 per cent of diabetics, but in only 10 per cent of non-diabetics. Patho-anatomically this process was characterized by marked proliferation of swollen endothelial cells, frequently obliterating the lumen further heavy deposits of PAS-positive hyaline fibrils were interlacing the proliferated endothelium. The PAS-positive fibrils failed to take the colloidal iron stain. The lamina elastica was single and unbroken. The process was found in almost 100 per cent of the vasa vasorum and the small periadventitial vessels of the big arteries, in 75 per cent of vasa nervorum, in 60 per cent of small skin vessels including capillaries and in some 40 per cent of the vessels of the muscle septa. The lesions were likewise found in diabetics without hypertension and in diabetics who never had had insulin.

In contrast, in arteriolo- and athero-sclerosis the deposit of hyaline material is not reticulated by endothelial cells, which are flat the PAS-positive material is scant and always staining positive with colloidal iron. Further the internal elastic membrane was frayed and reduplicated and did not stain well. The authors are of the opinion that they are able to discern between diabetes and non-diabetes.

Atherosclerosis in the big arteries was of the same frequency and intensity in the two materials, although the media in the sclerotic (non-diabetic) vessels tended to contain more calcium and bone. Extensive calcium deposits in small arteries were seen only in patients with hypertension moreover when hypertension existed, the characteristic differences were less striking

In arteries of the magnitude of digital arteries and bigger the diabetic lesion cannot be identified and vice versa the atherosclerotic process was not found in arteries of lesser magnitude in non-hypertensive individuals.

Material, technique and methods

The material for microscopical examination was gained by skin and muscle biopsy from the calves of 16 diabetics, from a toe of 2 diabetics and from amputated lower extremities from 4 diabetics (mid-thigh or below knee) In total the material came from 20 living diabetic patients (two patients were subjected to biopsy and afterwards investigation of the amputated leg) Besides, a small non-diabetic group of 4 patients with ischaemic impairment of the circulation of the lower extremities had biopsies of the legs done, and serves as a reference group.

The diabetic group[1] Of the 20 diabetics (12 males, 8 females) 18 came from medical Department C and 2 from surgical Department M. The material comprises all degrees of diabetes with respect to duration and

[1] The biopsies were done by the assistants to the surgical Department M (Head E. Hart Hansen, M D) the laboratory investigations were performed by the central laboratory (Head Per Lous, M. D) the eye examinations by E. Godtfredsen, M D and the neurological examinations by E. Skinhøj, M. D







Fig. 1 Photomicrographs of sections from skin and muscle biopsy from patients with diabetes.

) Small artery lesion of "endarteritis" type; the cellular layer of the intima is split-up by PAS-positive network of fibrils.

b) Capillary lesion. The basement membrane is thickened and stains PAS-positive.

) The vascular lesions are of an uneven, segmental distribution.

severity but as emerges from table I most patients had had diabetes for several years and showed complications. 2 patients had heir diabetes diagnosed at the present admittance (age 15 and 60 years) 4 patients had no complaints from their lower extremities and 16 had ulcerations or gangrene of these 11 belonged to the neuropathic type of lesion (Oakley et al. 1956 J Pedersen 1960) without claudication but with subjective and objective signs of alteration of sensibility with fading tendon reflexes, and in many cases with normal skin temperature and good pulsations of the arteries. 5 cases belonged to the "ischaemic" type with claudication, no pulses to be felt and with the patellar reflexes preserved. I few there was acute thrombosis of big artery

The control group 3 males, 51—69 years old, belonged to medical Department C, 1 female 59 years old to surgical Department M. The oral glucose tolerance test was completel normal as was haemoglobin. The sedimentation rate was nearly normal (7—12 mm/1 h.) All 4 patients had typical ischaemic claudication. By arteriography in three stop was shown i a. femoralis or a. iliaca ext. but in one in a. tibialis posterior only the patella- and achilles-reflexes were preserved and the skin sensibility was normal. Only 1 of the patients had an ulceration of big toe.

Technique The rather big biopsy was done under local anaesthesia in the surgical Department M on the back and lateral sid of the proximal half of the leg, at site far way from any ulceration or gangrene. The biopsy specimens were placed in physiological saline and immediately brought to the pathological laboratory as were amputated extremities.

Biopsy material. The tissue was fixed in 4 per cent aqueous solution of formaldehyde, the muscular biopsies af er one hour in physiological saline After embedding in paraffin, 20 serial sections were prepared from each block. The first 4 were stained with haema

Table I Data for the 20 diabetics (12 males and 8 females)

		Range	Average
Age (years)	Males	15—81	58.3 } 57.5
	Females	39—78	56.5
Duration of diabetes	(years)	0—31	12.4
Sedimentation rate	(mm/1 h)	6—153	51.2
Haemoglobin	(g/100 ml)	9.4—15.0	13.1
Serum cholesterol	(mg/100 ml)	129—491	258.0 (18 cases)
Protein in spinal fluid	(mg/100 ml)	44—99	65.4 (10 cases)
Protein in serum	(g/100 ml)	5.1—8.9	6.95 (18 cases)

Insulin treatment 18 cases. *Proteinuria 11 cases. Serum creatinine abnormal in 6 cases, max. value 3* mg/100 ml. Diabetic retinopathy 9 cases (none of the proliferative type).

In the small arteries and arterioles a characteristic lesion was found in 92 per cent of diabetics, but in only 10 per cent of non-diabetics. Patho-anatomically this process was characterized by marked proliferation of swollen endothelial cells, frequently obliterating the lumen further heavy deposits of PAS-positive hyaline fibrils were interlacing *the proliferated endothelium. The PAS-positive* fibrils failed to take the colloidal iron stain The lamina elastica was single and unbroken The process was found *in* almost 100 per cent of the vasa vasorum and the small periadventitial vessels of the big arteries, in 75 per cent of vasa nervorum, in 60 per cent of small skin vessels including capillaries *and in some 40 per cent of the vessels of the* muscle septa. The lesions were likewise found in diabetics without hypertension and in diabetics who never had had insulin

In contrast, in arteriolo- and athero-sclerosis *the deposit of hyaline material is not* reticulated by endothelial cells, which are flat the PAS-positive material is scant and always staining positive with colloidal iron. Further the internal elastic membrane was frayed and reduplicated and *did not stain well*. The authors are of the opinion that they are able to discern between diabetes and non-diabetes.

Atherosclerosis in the big arteries was of the same frequency and intensity in the two materials, although the media in the sclerotic (non-diabetic) vessels tended to contain more calcium and bone. Extensive calcium deposits in *small arteries* were seen only in patients with hypertension moreover when hypertension existed, the characteristic differences were less striking

In arteries of the magnitude of digital arteries and bigger the diabetic lesion cannot be identified, and vice versa the atherosclerotic process was not found in arteries of lower magnitude in *non hypertensive* individuals.

Material, technique and methods

The material for microscopical examination was gained by skin and muscle biopsy from the calves of 16 diabetics, from a toe of 2 diabetics and from amputated lower extremities from 4 diabetics (mid-thigh or below knee) In total the material came from 20 living diabetic patients (two patients were subjected to biopsy and afterwards investigation of the amputated leg) Besides, a small non-diabetic group of 4 patients with ischaemic impairment of the circulation of the lower extremities had biopsies of the legs done and serves as a *reference group*.

The diabetic group[1] Of the 20 diabetics (12 males, 8 females) 18 came from medical Department C and 2 from surgical Department M. The material comprises all degrees *of diabetes with respect to* duration and

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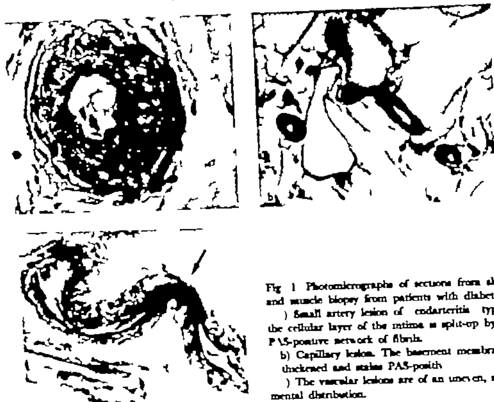


Fig 1 Photomicrographs of sections from skin and muscle biopsy from patients with diabetes.

) Small artery lesion of endarteritis type the cellular layer of the intima is split-up by PAS-positive network of fibrils.

b) Capillary lesion. The basement membrane thickened and stains PAS-positiv.

) The vascular lesions are of an uneven, segmental distribution.

severity but as emerges from table I most patients had had diabetes for several years and showed complications. 2 patients had their diabetes diagnosed at the present admittance (age 15 and 60 years) 4 patients had no complaints from their lower extremities and 16 had ulcerations or gangrene of these 11 belonged to the "neuropathic type of lesion (Oakley et al. 1956 J Pedersen 1960), without claudication but with subjective and objective signs of alteration of sensibility with failing tendon reflexes, and in many cases with normal skin temperature and good pulsations of the arteries. 5 cases belonged to the "ischaemic" type with claudication, no pulses to be felt and with the patellar reflexes preserved. I few there was acut thrombosis of big artery

The control group. 3 males, 51—69 years old, belonged to medical Department C, 1 female 59 years old to surgical Department M. The oral glucose tolerance test was completely normal as was haemoglobin The sedimentation rate was nearly normal (7—12 mm/1 h.) All 4 patients had typical ischaemic claudication. By arteriography in three a stop was shown i a. femoralis or a. iliaca ext. but in one in a. tibialis posterior only the patella- and achilles-reflexes were preserved and the skin sensibility was normal. Only 1 of the patients had an ulceration of big toe.

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Table II Skin- and muscle biopsies from the leg in 16 diabetics

Sex	Age (years)	Duration of diabetes (years)	Proteinuria	Diabetic retinopathy	Radiolog. calcification of arteries	Neuropathy of lower extremities	Small vessels			
							Skin		Muscle	
							Endarteritis	Accumulation of PAS positive material	*Endarteritis	Accumulation of PAS positive material
♂	81	4	+	+	+	+	+	+	+	+
♀	78	7	+	+	+	+	+	+	+	+
♂	78	10	+	+	+	+	+	+	+	+
♂	73	3	+	+	+	+	+	+	+	+
♀	66	30	+	+	+	+	+	+	+	+
♂	64	16	+	+	+	+	+	+	+	+
♀	60	(0)	+	+	+	+	+	+	+	+
♂	58	28	+	+	+	+	+	+	+	+
♀	57	18	+	+	+	+	+	+	+	+
♀	57	8	+	+	+	+	+	+	+	+
♂	52	15	+	+	+	+	+	+	—	−
♂	52	20	+	+	+	+	+	+	+	+
♀	41	31	+	+	+	+	+	+	+	+
♂	39	8	+	+	+	+	+	+	+	+
♀	39	4	+	+	+	+	+	+	+	+
♂	15	(0)	+	+	+	+	+	+	+	+

*Biopsy specimen very small (see discussion).

intensity from site to site. The nerves nearly always showed PAS-positive thickening of the arteriolar and capillary walls and in places fairly mild demyelinization unrelated to clinical signs of neuropathy

Discussion

The present study confirmed Goldenberg et al.s (1959) finding of characteristic angiopathy in the lower legs of diabetic subjects. It is of interest that now the angiopathy has been demonstrated in non-amputated extremities with no or only minor lesions. In 10 of our 16 patients the changes were demonstrated in skin-muscle biopsies. This incidence corresponds to that found by Goldenberg et al. in an amputation material *i.e.* approx. 63 per cent in dermal vessels and 48 per cent in muscular vessels.

Apart from involving the smallest vessels, this angiopathy is characterized by an uneven, segmental distribution which may be observed also within the same specimen (fig. 1 c). Owing to this fact, examination of even a large material need not show any changes. No wonder therefore, that Goldenberg et al. had to investigate numerous serial sections in order to find vascular changes in 92 per cent of their amputation series, and that in our biopsy series we did not find a

toxylin/eosin, PAS (Hotchkiss 1948) van Gieson/Hansen and Weigert's elastin stain. The remainder were PAS stained.

Material from amputated extremities The procedure was the same as described above. However the sections were fewer but from 6 different sites, int. al. from large vessels and nerves.

Results

In the microscopic examination we looked for the above mentioned changes described by Goldenberg et al. (1959) We paid particular attention to two characteristic forms of angiopathy

1) The marked intimal thickening in the small arteries which is caused by endothelial proliferation. In its presence the intima is not of a homogeneous appearance, but a split up cellular layer with a delicate, PAS-positive, network of fibres between the individual cells (endarteritis type, cf. fig 1 a)

2) PAS-positive, thickened basement membranes in the capillary walls and increased quantities of PAS-positive substance in arterioles and small arterial branches (cf. fig 1 b)

The differentiation from normal is based upon the experience of a large, highly varied material of muscle biopsies and especially on the study of biopsies in serial sections from the above mentioned small control series in which diabetes had been ruled out. In the control series we occasionally found PAS-positive thickening of capillary basement membranes, but always of slight extent. In rare cases, when doubt arose as to whether the thickness of the basement membrane in a diabetic subject exceeded that defined as normal the case was not classified as being of diabetic type. On a few occasions, endothelial proliferation showing the named characteristics was found in the control series too. In particular it may be impossible to distinguish inflammatory endarteritis from the diabetic changes described above, if the biopsy specimen has been obtained in the vicinity of an inflamed area (gangrene) This may be of significance especially in assessing biopsies from feet and toes.

Biopsy material. In table II the finding of the two characteristic vascular changes is marked + a more detailed grading being considered out of the question, at least in the present series. The fact is that the changes are of an uneven segmental distribution. In the same biopsy specimen a number of vessels may be entirely normal, while others are severely abnormal (cf. fig 1 c)

From table II it may be seen that vascular changes of the named types were found in 10 out of 16 patients. In 5 we found the characteristic endothelial proliferation in the small arterial branches as well as the thickening of the capillary walls. The remaining 5 had only PAS-positive thickening of the walls of capillaries and other small vessels.

Our small series does not appear to show any positive correlation of angiopathy involving the peripheral small vessels with the duration of diabetes, the presence of clinical signs of nephropathy neuropathy or retinopathy On the other hand, there was a correlation between chronological age and end arteritis.

Amputation specimens As already mentioned, we studied numerous preparations from 6 amputation specimens (2 toes and 4 legs) In all cases we found several changes of the classical arteriosclerotic type in the large vessels. In addition all showed diabetic angiopathy of the types described above, but varying in

organs (e g retinopathy glomerulosclerosis compared with angiopathy of the extremities) may be due mostly to differences in the anatomical structure of the different organs, including the vessels. In a recent publication moreover Blumenthal et al. (1961) claim to have found the typical proliferative lesions also in the retinal vessels of diabetics.

The demonstration of a generalized diabetic angiopathy will be of decisive significance for the interpretation and treatment of diabetes and its late complications. We shall, however restrict ourselves to brief comments on the extremity lesions.

As is well known, gangrene of the foot is far more common in diabetic than in non-diabetic subjects, in persons over 40 some 50—70 times more common. Moreover 60—80 per cent of patients with gangrene are suffering from confirmed diabetes (autopsy series, Bell 1957) Apart from its high incidence, gangrene shows another characteristic in diabetic patients. Gangrene of a patchy distribution may result from minimal injuries, and despite the presence of gangrene pulsation may be preserved in the arteries of the lower limbs. Unlike the findings in non-diabetics, the foot lesions are more rarely due to total or subtotal occlusion of the large vessels, although calcification of the large vessels of the lower limbs is far more common and occurs at an earlier age than in non-diabetic subjects. Particularly characteristic findings are chronic, penetrating pressure ulcerations — often associated with osteitis — and localized gangrene in a foot which is warm, even in the absence of infection, and which has preserved pulsation but a greatly impaired pain and temperature sense and extinguished tendon reflexes in the extremities. This clinical picture is due to neuropathy (*vide infra*) of sensory and sympathetic nerves which combined with impaired resistance to infection, is perhaps the most striking feature of the foot lesions in diabetics.

In diabetics, therefore, we must consider the following elements in the pathogenesis of gangrene and ulceration (cf. table III) (a) Calcification of the large vessels, (b) diabetic angiopathy of the small vessels, (c) neuropathy (d) reduced resistance to infection.

Calcification of the large arteries narrows their lumen, resulting in a reduced and slow blood flow even though pulsation is preserved.

In the feet there is an ample supply of small vessels of arteriolar size. Diabetic angiopathy involving these vessels and the capillaries impairs the peripheral blood supply even in the presence of a fairly adequate function of the large vessels.

When neuropathy involves the autonomous pathways, the result is functional impairment of capillaries and arterioles. Thus, the small peripheral vessels may be insufficient in two ways anatomically because of a lesion narrowing their lumen, and functionally because of a lesion affecting the vasomotor nervous system. (Cf. Bárány (1955) who concluded that the results of his experiments on capillary function in diabetics were best explained by anatomical damage to the capillaries without total occlusion, combined with neuropathy involving the autonomic pathways.)

Neuropathy of the sensory pathways gives rise to the impaired pain and temperature sense which often is responsible for the initial foot lesions, because injuries do not elicit the adequate reactions and the foot is far too long exposed to the harmful insult. If deep sensibility

Table III The effect of small vessel angiopathy in the lower extremities of diabetics

Vascular system		Nerve system (vasa nervorum)	
Large vessel (vasa vasorum) Calcification Stenosis	Small vessel PAS-positive deposits Stenosis	Autonomic pathway Degeneration Altered function of small vessels	Sensory pathway Degeneration Impaired pain- and temperature sense
	Insufficient blood supply		Initial foot lesion

positive correlation with the duration of diabetes or with the presence of clinical signs of neuropathy nephropathy or retinopathy Likewise the positive correlation which we found between the type of endarteritis and chronological age in skin muscle biopsies we also dare not interpret at the present stage of knowledge. Obviously a skin muscle biopsy is not suited for clinical assessment of the diagnosis, prognosis or therapeutic effect — especially as negative findings do not rule out vascular changes in other areas. This point is illustrated by one of our cases. While no alterations of definite diabetic type were seen in the rather small biopsy material massive alterations of both types were seen in muscles and nerves from the amputated leg Failure to make use of serial sections and PAS staining may be assumed to explain why the typical changes have not been more commonly observed previously

The nature of the accumulated pathological substance is not definitely known. It probably does not represent acid mucopolysaccharides, as the deposited PAS-positive substance does not react with colloidal iron (Goldenberg et al. 1959) In this connection, it is of interest that PAS-positive diffuse and nodular deposits in renal glomeruli in diabetics were neither acid mucopolysaccharides nor glycogen but probably neutral polysaccharides (Hatch et al. 1961)

The changes are hardly specific, but probably characteristic of diabetes, especially in their localization to the small vessels. Endothelial proliferation like that of diabetes may be seen e g in non-diabetics in infected areas, for instance in the vicinity of a gangrenous toe, and also in syphilis and immuno-allergic diseases (Goldenberg et al. 1959)

These findings strongly support the concept of generalized involvement of the small vessels in diabetics — a diabetic angiopathy In comprehensive studies of diabetics with neuropathy and nephropathy respectively Fagerberg (1959) and Aa. Chr Thomsen have demonstrated similar changes, i e accumulation of PAS-positive material, mural thickening and narrowing of the lumen in the vasa nervorum and the small renal vessels. Moreover Aagenæs & Hågensen (1959) found identical changes in small vessels from the synovial membrane derived from a diabetic Charcot joint. Lastly Aagenæs & Moe (1961) have recently published light and electron microscopic studies of capillaries from the digital pulpae of diabetics which showed segmental accumulation of PAS-positive material in the periendothelial layers. Thus very strong evidence of an angiopathy typical of diabetes has accumulated during the last 2 years. That this angiopathy does not manifest itself morphologically in the same way in all

lesion, is so important to the diabetic with this type of "neuropathic" foot lesion.

In cases of foot lesions due mainly to ischaemia caused by subtotal or total occlusion of a large artery the chances of repair are considerably poorer than in non-diabetics. Establishment of a by-pass, e. g. on account of a stop in the femoral artery is seldom indicated, int. al. because the popliteal artery is nearly always so involved that the prosthesis cannot be properly fixed. In the event of a more acute thrombosis or embolism, operation may be considered, but its permanent result cannot be expected to be particularly favourable, since the angiopathy of the small vessels persists.

Although the prognosis of a "neuropathic" foot lesion — even a large one involving ulceration, osteitis and inflammation — is not too bad, the prospects at longer sight and in the presence of ischaemic lesion are very gloomy indeed. At present, therefore, the efforts must be directed at more effective prevention of the angiopathy i. e. better management of the diabetes and, when angiopathy has arisen, better prophylaxis against the development of the lesions.

Summary

In a series of 20 diabetics, investigation of serial sections of skin-muscle biopsies (16 patients) and material from amputated legs (4 patients) and toes (2 patients) confirmed that diabetics suffer from characteristic, diffuse, but segmentally distributed, angiopathy of the small vessels.

This diabetic small-vessel angiopathy is assumed to be the primary factor in the common occurrence of extremity lesions in diabetics. In addition to involving a poor peripheral blood supply the small-vessel angiopathy gives rise to neuropathy (vasa nervorum) and presumably also to calcification of the large arteries (vasa vasorum). It cannot be ruled out that this small-vessel angiopathy is also an important factor in the reduced resistance to infection.

The clinical importance of making a distinction between "neuropathic" and "ischaemic" extremity lesions is pointed out.

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is involved, diabetic arthropathy of the tarsus may develop corresponding to the classical Charcot joint.

Regardless of which vascular area is most affected, reduced resistance to infection is a serious complication.

This leads us to the question whether the angiopathy of the small vessels can give rise to the other three elements. The cause of the calcification in the big arteries has been a subject of dispute arteriosclerosis or diabetic angiopathy Despite biochemical evidence of differences from arteriosclerosis in the lipid pattern and in the cholesterol/phospholipid ratio (Lundbæk 1954) a diabetic angiopathy is not generally accepted because the vascular changes must be called morphologically non-specific, at least judging by the histological methods used so far As already emphasized, Goldenberg et al. (1959) found the small-vessel angiopathy with particular frequency (almost 100 per cent) in the vasa vasorum and in the periadventitial vessels It must be considered a fact that angiopathy of the vasa vasorum impairs the blood supply to the walls of the large vessels and it is likely — but of course not proved — that this gives rise to premature degeneration and calcification. Diabetic angiopathy of the small vessels might be the cause of calcification in the large vessels.

The studies of Fagerberg (1959) and Goldenberg et al. (1959) appear to have proved that the diabetic neuropathy of the extremities involving degeneration of nervous tissue, reduction in the number of non myelinized (vasomotor) nervous fibres, as well as demyelinization of nerve sheaths (Martin 1953 Fagerberg 1959) is due to the typical diabetic angiopathy of the vasa nervorum.

The anatomical changes in the small vessels, together with the neuropathy must be assumed to play a role in the reduced resistance to infection. The thickened vessel wall must give poorer conditions for exchange of substance between blood and tissue, and the blood flow is impaired

Diabetic angiopathy of the small vessels must be considered the cause of the neuropathy and probably it is responsible for the calcification in the large vessels. Furthermore it cannot be ruled out that it is an important factor in the reduced resistance to infection.

Accordingly the small vessel angiopathy may be considered the origin of extremity lesions in diabetics. As outlined above, however the clinical manifestations of extremity lesions may be manifold. This is due to varying combinations of the above-mentioned four elements the small-vessel angiopathy being of varying degree and of segmental distribution.

These considerations are of clinical significance. Although *per se* all extremity lesions are angiopathic, a particular lesion can nearly always be classified as being predominantly of the "neuropathic" or of the ischaemic type (Oakley et al. 1956, J Pedersen 1960) Most ulcerations and gangrenous lesions are due to a combination of severe neuropathy and involvement of the small vessels in the peripheral tissues while at the same time the blood supply through the large arteries is fairly sufficient. In other words, the diabetic develops his lesion at a time when the blood supply through the vessels is not sufficiently impaired to lead to gangrene or necrotizing ulcers in a non-diabetic subject. This is the reason why conservative management including conservative surgery and prophylactic measures against the formation of the

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Three Cases of Chronic Idiopathic Jaundice

Clinical and Histological Observations

By

MARTTI LEHTINEN and PER FORTELIUS

Chronic idiopathic jaundice was first described, independently by two groups of research workers, Dubin and Johnson, and Sprinz and Nelson (2, 7) The clinical picture is characterized by chronic or intermittent icterus from increase in conjugated bilirubin, frequent familial occurrence, exceptional bromsulphalein metabolism, and the centrolobular presence of brownish pigment in the parenchymal cells of the liver Opinions advanced in the literature on the quality of the pigment are contradictory (1 7 8 9)

In 1958 Dubin (1) collated the material on the 50 cases which had been described up to that date. Several individual cases of chronic idiopathic jaundice have since been reported, and families with several members affected with the disease have also been described (5 9) The incidence of the disease among the general population is not yet known with certainty

The three cases of chronic idiopathic jaundice to be presented here were diagnosed in Maria Hospital within six months. Two of the patients are brothers. A typical clinical feature is the long duration of the jaundice which has averaged 50 years. Particular attention has been devoted to the histochemical study of the pigment.

Case reports

Case 1 Male, born 1909 Occupation: plumber

From the age of 13 the patient has been permanently yellow the degree of icterus varying greatly all diseases, including slight colds, aggravating it. The yellow appearance has never entirely disappeared, and the urine has been consistently dark in colour The patient has experienced occasional pains in the right upper abdomen, particularly since a cholecystectomy in 1934. In the 1930' the patient consumed alcohol more or less regularly but subsequently only occasionally I 1945 he was treated in hospital for abdominal pain

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Table 1. The most important clinical findings and the results of laboratory and X-ray examinations in the [illegible] *cases of chronic idiopathic jaundice*

	Case 1	Case 2	Case 3
Clinical features			
Jaundice			
Age when first noted	15 yrs	12 yrs	8 yrs
Duration to date	39	49	55
Familial occurrence	Present	Present	None
Hepatomegaly	Slight	Slight	Slight
Laboratory studies			
Serum total bilirubin	3.9 mg %	4.6 mg %	3.7 mg %
Serum conjugated bilirubin	3.5 mg %	4.0 mg %	3.3 mg %
Serum non-conjugated bilirubin	0.4 mg %	0.6 mg %	0.4 mg %
Meulengracht' icterus index	1 18	1 20	1 17
Bilirubinuria	+	+	+
Bromsulphalein retention	14 %	20 %	23 %
Szokc reaction	1 76	1.72	1.50
Thymol test	+	—	—
GOT	8 U	14 U	22 U
Alkaline phosphatase (BL-unit)	1.50	1.76	1 45
Cholesterol	240 mg %	198 mg %	210 mg %
Electrophoresis	Normal	Normal	Alfa 2 augment.
Prothrombin per cent	90 %	90 %	93 %
Radiological studies			
Oral cholecystography	— (operated)	Nonvisualization	Poor visualization
I. cholangiography	Poor visualization	Poor visualization	Poor visualization

been dark. All illnesses, including common colds, physical effort and lack of sleep had caused the yellow colour to deepen. She had seldom consumed alcohol and had noticed no effect on the icterus. Despite the jaundice she had as a rule felt perfectly fit and had been capable of all manual work.

The patient was treated in 1944 at Maria Hospital for erysipelas and phlegmone of the left leg, and the icterus index values recorded were 1 60 and 1 32. In the 1950's, the patient was frequently examined at the Out-patient Department of the hospital for post-traumatic arthrosis of the right knee, and on these occasions slight icterus was noted regularly index 1 15—1 20. In April 1961 the patient contracted influenza, with fever cough, and pain in the extremities. A week later the condition was aggravated by dysuria and pain in the lower and central abdomen. Sigmamycin therapy had brought transient relief. A few days after the termination of therapy the patient contracted septic fever and was admitted to the Medical Department of Maria Hospital. Her temperature was 39° C and the leukocyte count 14,000/mm^3 urine analysis showed leucocytes and bacteria, and a rod bacterium of E. coli type grew on culture. On electrophoresis, the alpha 2 fraction had increased (19 7 %) The clinical diagnosis was pyelonephritis. Therapy consisted of antibiotics according to bacterial sensitivity determinations (first streptomycin and later chloramphenicol) continued with longterm Madribon course. During therapy the patient's urine had become sterile, and the fever and subjective symptoms had disappeared. The erythrocyte sedimentation rate has continued to diminish and was last recorded t 47 mm/1 hour

Examination carried out for icterus revealed increase in conjugated bilirubin, and liver function tests were normal with the exception of heavily disturbed bromsulphalein test and a change in electrophoresis which was obviously due to infection. Nothing suggestive of ac

and jaundice the diagnosis was chronic hepatitis with incipient cirrhosis. In 1952 the patient was again admitted to hospital for fever and articular pain. The findings included a distended liver slight icterus, and normal liver function tests. The articular symptoms were treated with analgesics and physiotherapy and the hospital diagnosis was again chronic hepatitis.

In 1954 the gallbladder was poorly visualized by cholecystography In the same year a cholecystectomy was performed at Maria Hospital for a suspected gallbladder disease. No calculi were found in the gallbladder which on histological examination looked normal, but during operation, the abnormally dark colour of the liver was noted. The biopsy specimen taken showed capsular fibrosis. Post-operatively the patient frequently attended the Outpatient Department for follow-up He was admitted to the Medical Department of Maria Hospital in 1957 and was subjected to laparoscopy which, however remained technically incomplete because of adhesions following the cholecystectomy An attempt to take a biopsy specimen also failed. The diagnosis made was cirrhosis, and treatment consisted of numerous liver extract injections. The patient's general condition continued to be good, and he was capable of heavy manual work.

In 1960 the patient was admitted to the Medical Department of Maria Hospital for severe precordial pain ECG revealed an extensive posterior wall infarction, which was treated in the usual way i. e. by rest and anticoagulants. After a month the patient was discharged but had another infarction at home and was re admitted to hospital. He is continuing with anticoagulant therapy and visits the Outpatient Department regularly for follow up. The slight icterus was at this stage overshadowed by the heart disease. In October 1960 when the patient again had a heart check-up at the Outpatient Department, attention was attracted by the incompatibility of the clinical picture with the diagnosis of cirrhosis. It was discovered that the patient's brother had also been yellow since childhood. The liver biopsy specimen taken during cholecystectomy in 1954 was re examined and found to show pigment typical of chronic idiopathic jaundice. The laboratory tests listed in table I supported this diagnosis.

Case 2. Male, born 1900 Occupation stonemason.

The first patient's elder brother he was admitted to the Medical Department of Maria Hospital for coronary disease after the final diagnosis of case 1 had been confirmed. He reported that he had been yellow from the age of 12. The yellow colour had persisted with varying intensity and the urine had regularly been dark brown in colour The patient had noted that physical effort increased the icterus. He took alcohol approximately twice a month. It did not affect the yellow colour The patient had never felt any abdominal pain. Throughout his life he had done heavy manual work, lately as a stonemason. He had never consulted a doctor for icterus, but had once been hospitalized for 5 days for a fever In recent months he had noted dyspnoea and precordial pain on strenuous effort, and was admitted to hospital for a severe attack. ECG showed a left bundle branch block the clinical course with transient fever and a temporary increase in erythrocyte sedimentation rate, the leukocyte count and serum GOT level suggested myocardial infarction.

The patient was found to have slight icterus and slight hepatomegaly was noted. In laboratory investigations, the liver function tests gave values equalling those of the patient's brother (table I) The diagnosis of chronic idiopathic jaundice was confirmed by the liver biopsy taken according to the Menghini method.

According to the brothers, other members of the family were not known to be icteric. Since their relatives are scattered throughout Finland they could not yet be reached for examination.

Case 3. Female born 1900. Janitor's wife.

The incidence of tuberculosis in the patient's family had been high She did not know that any member had had icterus. One of the patient's sisters was alive and she had been operated on for carcinoma of the uterus. The patient's son and grandson live in Canada they are healthy

The patient's jaundice was probably first noted at the age of 8 although she remember no details. She said that she had always been slighty yellow wh ch was particularly noticeable in the sclerae. The urine had usually

Table I. The most important clinical findings and the results of laboratory and X-ray examinations in the three cases of chronic idiopathic jaundice

	Case 1	Case 2	Case 3
Clinical features			
Jaundice			
Age when first noted	13 yrs	12 yrs	8 yrs
Duration to date	39	49	55 »
Familial occurrence	Present	Present	None
Hepatomegaly	Slight	Slight	Slight
Laboratory studies			
Serum total bilirubin	3.9 mg %	4.6 mg »	3.7 mg %
Serum conjugated bilirubin.	3.5 mg %	4.0 mg %	3.3 mg %
Serum non-conjugated bilirubin.	0.4 mg %	0.6 mg %	0.4 mg %
Meulengracht icterus index	1 18	1 20	1 17
Bilirubinuria	+	+	+
Bromsulphalein retention	14 %	20 %	23 %
Stoke reaction	1.76	1.72	1.50
Thymol test	+	—	—
GOT	9 U	14 U	22 U
Alkaline phosphatase (BL-unit)	1.50	1.76	1.45
Cholesterol	240 mg %	198 mg %	210 mg %
Electrophoresis	Normal	Normal	Alfa 2 augment.
Prothrombin per cent	90 %	90 %	93 %
Radiological studies			
Oral cholecystography	— (operated)	Nonvisualization	Poor visualization
I. cholangiography	Poor visualization	Poor visualization	Poor visualization

been dark. All illnesses, including common colds, physical effort and lack of sleep had caused the yellow colour to deepen. She had seldom consumed alcohol and had noticed no effect on the icterus. Despite the jaundice she had as a rule felt perfectly fit and had been capable of all manual work.

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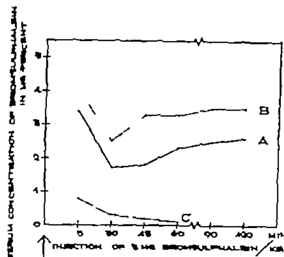


Fig 1 Bromsulphalein disappearance curve A = case 2 B = case 3 C = normal subject

celerated red cell haemolysis was noted. Cholecystography and intravenous cholangiography gave poor visualization of the extrahepatic biliary ducts and the gallbladder The histological study of a biopsy specimen taken with Menghini needle confirmed the diagnosis of chronic idiopathic jaundice.

Results

Clinical and laboratory findings

Table I lists the principal clinical findings and the results of laboratory and X ray tests for all three patients. Fig 1 gives the bromsulphalein disappearance curve for cases 2 and 3 The phenomen first described by Mandema et al (5) typical of chronic idiopathic jaundice is clearly visible in fig 1 The concentration of pigment in the serum first declines in the normal way to a certain saturation point. Presumably as a result of disturbed secretion into the bile of pigment conjugated in liver cells, some bromsulphalein returns to the serum causing a new rise in its concentration

It might be added that none of the patients showed changes in the blood picture signs of increased haemolysis, demonstrated splenomegaly or portal hypertension

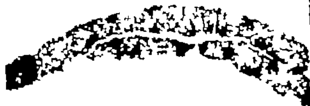
Histology and histochemistry

The specimens from the three cases were obtained and treated as follows

In the case of the first of the two brothers investigated, formalin fixed, paraffin embedded material, seven years old, was available. The specimens had been obtained in connection with a cholecystectomy and consisted of a piece of liver tissue and another of the gallbladder wall. In routine preparations of the gall bladder wall nothing clearly pathological was observed. The specimen from liver of the second brother was obtained by needle biopsy and fixed in 10 per cent buffered neutral formalin Part of it was sectioned in an ordinary freeze-microtome. The greater part was embedded in paraffin. In the case of the third patient, the woman, a needle biopsy was also performed. The resulting liver fragment was, however too small for division and processing in the freezing microtome was therefore omitted. Fixing and embedding followed as for the other biopsy specimen.

The surgically obtained liver sample being the largest allowed the best histological evaluation The histological pictures in preparations from both the brothers were, however apparently similar predominantly and often distinctly in a centrilobular fashion reaching to about half the lobular radius, a golden brown coarsely granular pigment being apparent on microscopy This corresponds to a grade 2 change according to Wolf et al (9) The pigmentation was the only remarkable histological feature. The lobular architecture was not disturbed.

In case 3 only small and fragmentary sections with parts of the liver lobules were seen in the preparations. Somewhat patchy areas with aggregations of the same type of intracellular parenchymal



2. Case 2. Needle biopsy spec-
en patchy central pigmentation
arly visible Schmorl reaction.
jective 2 1/2 final magnifica-
n 20



Fig. 3. Case 1 Surgical biopsy specimen distinct accumulation of pigment around the two central cins. Azur-eosin. Objective 10 final magnification 100

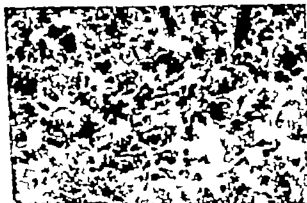


Fig 4 Case 3. Needle biopsy specimen coarsely granular hepatocellular pigmentation around central vein. Giemsa. Objective 45 final magnification 350

pigment as shown by the other two cases were present in abundance. Around two recognizable central veins a rather heavily pigmented zone was observed. The oriented liver cell trabeculae pointed to a normal structure of the lobules.

In all three liver specimens there was no evidence of extracellular cholestasis. The fairly light brown pigment collected into coarse granules showed no similarity with the usually rather finely granulated dark deposits of intracellular bile pigment. The Gmelin test for bilirubin was negative (6) Neither inflammatory changes nor significant liver cell degeneration were found. In the liver sections from the cholecystectomized brother a thick fibrotic capsule and a slight fibrosis in some

periportal areas were seen conforming to the initial histopathological diagnosis. In the light of later facts, however these findings seems unimportant.

In Masson s trichrome stain in v Gieson and Mallory — azan stained sections the finely granular pigment of type II as distinct from the coarser granules of type I described by Wegmann et al. (8) could not be identified with certainty nor was it apparent in differently treated sections. The rather coarse granules which we examined corresponded quite well to the pigment type I of Wegmann et al. It was not apparent extracellularly or in mesenchymal cells.

The clinical and routine histological findings strongly suggested Dubin-Johnson s disease in the three patients. To confirm the diagnosis and to compare the histological observations with those described in papers on the subject, a number of histochemical tests were carried out on the liver specimens. In spite of the long storage of the oldest specimens (7 years) the tests performed on sections from paraffin embedded liver samples from the three patients produced similar results.

The pigment granules showed no birefringence, a finding also observed by Dubin (1) indicating amorphous aggregations. The dull brown fluorescence quoted by Dubin (1) and Wolf et al (9) was not confirmed. On the other hand, the granules were visible as dark deposits against a weak brown fluorescence of the liver cells mentioned by Lillie (3) and Pearse (6) Staining with Fettrot 7 B (Ciba) as a saturated solution in isopropanol did not colour the pigment granules in the paraffin sections Frozen sections from the needle specimen of the second investigated brother showed no colouring with Fettrot of the pigment aggregates. Some red-staining vacuoles and small grains could be seen in a fashion typical of normal liver tissue.

Perl s test for ferric iron was negative in the pigment granules of all liver specimens. Small quantities of fine blue grains were observed in some liver cells, and in some endothelial and mesenchymal cells, as is usual in the normal liver

Ziehl Nielsen s acid fast staining reaction for lipofuscins according to Pearse (6) was negative for all liver sections. In the gallbladder wall of the cholecystectomized brother positive granules in smooth muscle cells were seen serving as a positive control and apparently indicating lipofuscin.

The Schmorl test for ferricyanide reducing groups, including melanin and lipofuscin was strongly positive in the pigment granules of all liver specimens.

After staining with Lillie s azur-eosin at pH 5 6 the pigment aggregates showed strong azurophilia. Chromaffinity could not be demonstrated, since the fixation was in formalin and postchromation in our experience, produces doubtful results. This has also been pointed out by Lillie (3) The periodic acid Schiff reaction was mostly negative in granules, although some intermixing with red granules occurred in the preparations not treated with saliva. In the diastase treated sections, there were practically no positive granules. This observation indicates an intermixing of glycogen in some of the pigment granules.

The liver pigment in question exhibited affinity for Fe^{+} which is regarded by Lillie (4) as quite specific for melanin

Comments

Three cases of chronic idiopathic jaundice have been described. The diagnostic criteria for all were increase in

conjugated bilirubin, disturbed bromsulphalein metabolism and the presence in the liver of coarsely granulated pigment of melanin type. All these findings reflect the disorder in the secretory function of liver cells, which is characteristic of the disease. The exact mechanism of the disorder is unknown.

A special feature in the cases reported is the long duration of icterus, ranging from 39 to 53 years. In Dubin's series, the duration of icterus exceeded 30 years in only 5 out of 50 cases. Only one of the present patients, case 1, had felt occasional abdominal pain in the region of the liver obviously connected with chronic idiopathic jaundice. He had also been hospitalized for icterus, submitted to poorly indicated cholecystectomy to long-term therapy with injections of liver extract, and unsuccessful laparoscopy before diagnosis was clear.

All the patients reported that febrile diseases and heavy physical effort brought about an increase in icterus, while none of them noticed any similar effect on alcohol consumption.

At the moment of diagnosis of chronic idiopathic jaundice, none of the patients was in hospital for icterus: cases 1 and 2 had been admitted for coronary disease, case 3 for pyelonephritis.

Centrilobular coarsely granular liver pigment predominated in liver samples from three patients with clinical evidence of Dubin-Johnson's disease. Obviously it was not bile pigment and gave negative reactions for iron and lipides. There was some evidence which suggested the melanin nature of the pigment: the granules showed an affinity for ferrous ions (4) and there was a lack of fluorescence on irradiation with ultra violet rays. Of less significance were the positive results of tests for both melanins and lipofuscins, the Schmorl reaction and the argentaffinity combined with negative results for more specific reactions for lipofuscins.

Evidence against the lipofuscin nature of the pigment investigated was provided by the negative stains for lipids and for acid-fast lipofuscins, and by the negative periodic acid Schiff reaction. None of these tests entirely excludes the group of lipofuscin pigments, but taken together they cannot be overlooked. Lipofuscin should, in addition, be fluorescent, but as mentioned above this condition could not be proved in our cases. Our observations regarding the lipofuscin nature of the atypical liver pigment found in Dubin-Johnson's disease are somewhat contradictory to those of Dubin (1) but agree with those of Wolf et al. (9) except for the fluorescence of the granules.

Our observations in support of melanin in the coarse granules do not entirely accord with the findings of Wegmann et al. (8) who regarded a finely dispersed pigment (type II) as more important. They also claimed to have isolated a similar pigment from the spleen of a patient suffering from Dubin Johnson's disease, and to have shown, by infrared spectrophotometry that it was melanin. A number of histochemical observations on the liver of the same patient were presented, and these indicated the pigment type II to be of melanin nature. In view of the observations on the liver pigment differing to some extent in cases of Dubin-Johnson's disease, it appears that the observed pigment aggregates might vary in composition. Even if the fundamental metabolic disturbance is regarded as similar, secondary admixing of varying substances according to individual physiological or pathological states would have to be considered.

The laboratory findings and the histo

logical picture of the liver are surprisingly similar for the two brothers of the present series despite the difference in their medical histories the findings are very nearly identical The dramatic concurrence of the disease of these brothers is also reflected in the observation that both suffer from a serious coronary insufficiency

Summary

The authors report their clinical and histological observations on 3 cases of chronic idiopathic jaundice. Two of the patients were brothers. The disease had in all three cases originated in childhood, and the duration of icterus to date ranged from 39 to 53 years. The two brothers suffered from coronary disease, while the third (female) patient with no familial occurrence, was hospitalized for pyelonephritis.

All the patients showed slight icterus and slight hepatomegaly Only one complained of occasional pain in the hepatic region.

The laboratory findings were very similar in all three cases apart from conjugated hyperbilirubinaemia and disturbed bromsulphalein metabolism the liver function tests were for the most part normal. In all the cases, the visualization of the extrahepatic biliary ducts and gall bladder on cholecystography and cholangiography was poor or non-existent.

The histological picture was similar for all the cases. Centrolobular pigmentation was observed, and on histochemical examination proved to be of the melanin type.

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From the Institute of Physiology University of Helsinki, Helsinki, Finland

The Effect of Weight Reduction on Pulse Rate, Blood Pressure, Ventilation and Oxygen Consumption during Rest and in Connection with Muscular Exercise

By

Martti Kotilainen and Anja Kotilainen

The effect of loss of weight on pulse rate, blood pressure, ventilation and oxygen consumption has been investigated by Proger and Magendantz (1936) in persons suffering from cardiac insufficiency by Master et al. (1936) in persons having coronary artery diseases, and by Master et al. (1942) in a series of five healthy experimental subjects. They found a reduction of oxygen consumption, pulse rate, blood pressure and cardiac output, after weight reduction. The papers of Martin (1952) and Fletcher (1954) may also be mentioned.

The changes in the respiratory functions produced by loss of weight have been investigated by Saltzano et al. (1958) who found that no significant change occurs in the minute respiratory volume at rest. On the other hand decrease of the oxygen consumption during rest as well as under muscular exercise is observed in the majority of cases.

All above mentioned studies lack a control series. In some of them, moreover the subjects have some disorder of the cardiovascular system.

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The purpose of the recent work was to study the effect of loss of weight on pulse rate, blood pressure, ventilation and oxygen consumption during rest and muscular exercise in a series of persons who are healthy but for their obesity. The results obtained are compared with control series and the results are analyzed for correlation between the degree of loss of weight and the observed changes.

Material and methods

The weight reduction group proper consisted of 13 women and 11 men. The women were on an average 44 % overweight before and 26.5 % after dieting, the men 36 % and 19 %, respectively. Normal weights used according to tables of the Metropolitan Life Insurance Company. The subjects in the weight reduction group had no cardiorespiratory disorder observable by clinical examination.

The so-called diet group comprised seven subjects. They followed the same diet plan as the weight reduction group proper but they were re-examined one week after commencement of the diet, before any consider

Table I

	Weight reduction group		Diet group	Control group
Resting pulse rate	Women	Men		
Cases showing decrease /increase/ no change	8/—/5	8/2/1	5/2/—	4/2/1
Mean decrease, beats per min	6.5 ± 1.8	5.5 ± 2.5	3.0 ± 2.6	1.1 ± 1.8
P	< 0.01	> 0.1	> 0.1	~ 0.1
Exercise pulse rate				
Cases showing decrease /increase/ no change	11/2/—	8/1/2	4/1/2	6/1/0
Mean decrease, beats per min.	10.8 ± 3.1	9.9 ± 3.6	2.3 ± 1.9	5.3 ± 2.8
P	< 0.01	< 0.05	> 0.1	> 0.1
Blood pressure syst./diast.				
Cases showing decrease /increase/ no change[1]	5/—/8	2/—/9	2/—/5	3/— 4
Mean decrease, mmHg	7.3 ± 4.4/6.6 ± 2.1	8.0 ± 3.8/4.0 ± 2.1	8.4 ± 2.7/4.7 ± 2.1	6.5 ± 3.4 4.8 ± 2.5
P	> 0.1/< 0.01	> 0.1/> 0.1	< 0.05/> 0.1	> 0.1 > 0.1

A change was recorded if the difference was 3 beats per min. or more.

A change was recorded if the difference was 10 mmHg in diastolic pressure or more.

able loss of weight had occurred. Seven more subjects constituted the actual control series. Their food intake was not controlled during the time between examinations. The weight reduction programme had a duration of three months and the recommended diet contained about 800 calories per day

The following experimental arrangement was employed The subjects arrived fasting, early in the morning to the examination After an initial 20—30 minutes period of rest in recumbent position, with the rubber mask already fitted over the head, pulse rate and blood pressure readings at rest were taken by auscultation three times the lowest reading was taken to be significant. A mercury manometer was used to take the blood pressure, its readings being controlled repeatedly during the test period. The cuff had a width of 13 cm.

After these initial measurements the subject breathed into a Douglas bag during ten minutes. The volume of the collected gas was determined with the gasometer and a sample was analyzed for its CO_2 and O_2 content by Haldane's method

After the resting values had been measured, the subject performed Master's two-step test, which is standardized with respect to age sex, and weight however the same degree of exercise was applied also after loss of weight. As it was found that the increase of pulse rate and ventilation was fairly insignificant in most of the subjects after the conventional step test of 1 1/2 minutes duration according to Master the test was repeated after five minutes rest, increasing its duration to 3 minutes so that more appreciable differences might be elicited. This presented the further advantage that the first time accustomed the

Table II

	Weight reduction group		Diet group	Control group
	Women	Men		
Resting ventilation				
Cases showing decrease /increase/ no change[1]	5/3/4	2/5/3	1/2/4	1/1/5
Mean decrease, l/10 min.	3.3	— 0.4	— 0.7	0.2
P	> 0.1	> 0.1	> 0.1	> 0.1
Ventilation during exercise				
Cases showing decrease /increase/ no change[2]	11/—/2	7/—/3	3/1/3	4/1/3
Mean decrease l/3 min.	10.3 ± 1.5	15.7 ± 4.7	3.5 ± 2.8	3.9 ± 2.2
P	< 0.001	< 0.01	> 0.1	> 0.1
Resting oxygen consumption				
Cases showing decrease /increase/ no change[3]	3/1/9	3/—/7	1/—/6	1/2/4
Mean decrease, ml/10 min.	68 ± 66	155 ± 48	30	— 30
P	0.1	< 0.02	> 0.1	> 0.1
Oxygen consumption during exercise				
Cases showing decrease /increase/ no change[3]	11/—/2	10/—/—	2/1/4	3/—/4
Mean decrease, ml during 3 min. exercise	458 ± 83	843 ± 142	171 ± 64	150 ± 88
P	< 0.001	< 0.001	< 0.05	> 0.1

[1] A change was recorded if the difference was 5 l per 10 min. or more.
[2] A change was recorded if the difference was 5 l per 3 min. or more.
[3] A change was recorded if the difference was 200 ml per 10 min. or more.

subject to ascend the steps with Douglas bag on his back. Ten seconds after termination of the test the "exercise pulse rate" was taken every 15 seconds until the level at rest had been regained. The "exercise pulse rate" observed during the period between 10 and 70 seconds after termination of the exercise was used in the subsequent calculations. The air exhaled during the three-minute test was again collected in the Douglas bag, the gas quantity and its CO_2 and O_2 contents were determined.

As it was exceedingly difficult to time the blood pressure measurements under these conditions exactly on the second with given point of time after termination of the exercise, reading the blood pressure after exercise had to be abandoned.

Results

The average weight reduction in three months was 10.6 kg (12.1 %) for the women and 13.2 kg (12.8 %) for the men in the weight reduction group.

The results of the examinations are shown in tables I and II.

Discussion

With respect to the resting pulse rate attention is drawn to the fact that it shows a significant decrease only with the women in the weight reduction group. Buskirk et al. (1955) in their investiga-

tion on the relationship between obesity and pulse rate, noted that the resting pulse rate of untrained persons is the higher the higher the relative degree of obesity But this is not true in the case of trained subjects The higher degree of training in men may be responsible for the fact that the decreasing tendency of the resting pulse rate is not as distinct in the men as in the women.

The exercise pulse rate is lower after weight reduction and a distinct difference in comparison with both control groups exists.

It has been demonstrated in numerous investigations (Wendkos and Rossman 1943 Trout et al. 1956) that the indirectly measured blood pressure values are higher the greater the thickness of the fat layer around the upper arm. However Fletcher (1954) reports on the basis of his test series a statistically significant correlation between loss of weight and decrease of blood pressure even after loss in girth of the upper arm has been accounted for

Although no statistically significant decrease of blood pressure can be observed in the present material, lower values have as a rule been recorded both after weight reduction and at the second examination in the control groups It is therefore likely that the reduction of blood pressure readings are simply due to familiarity with the testing procedure.

The changes in resting ventilation were random in character in all groups A decrease bordering statistical significance in resting oxygen consumption occurred in the men of the weight reduction group In the case of the women the high variations rendered the decrease statistically non-significant, so there is probably no difference between sexes in this respect The differences in comparison with the control groups still seem distinct enough.

The most distinct changes have occurred in ventilation and oxygen consumption during exercise. Nielsen (1937) has shown that ventilation and oxygen consumption during work increase in proportion with the quantity of work. The statistically highly significant reduction of ventilation and oxygen consumption during exercise in the weight reduction group was found to be equivalent in its order of magnitude to the actual decrease of work implied by the loss of weight. In the diet group there occurred a statistically significant decrease of the oxygen consumption during exercise, but it was far less than that in the weight reduction group

The correlations between the percentage loss of weight and the various functions were studied. The following correlation coefficients were found.

Per cent loss of weight x.

resting pulse rate	$r = 0.04$ $P > 0.1$
exercise pulse rate	$r = 0.26$ $P > 0.1$
blood pressure	$r = 0.005$ $P > 0.1$
resting ventilation	$r = 0.28$ $P > 0.1$
ventil. during exercise	$r = 0.46$ $P < 0.05$
resting O consumption	$r = 0.02$ $P > 0.1$
O_2 consumption during exercise	$r = 0.42$ $P < 0.05$

As may be seen the reduction in ventilation and oxygen consumption during exercise were the only quantities to correlate with the percentage loss of weight The presence of a correlation in these two instances is readily understandable, as loss of weight implies reduction of the work load if the latter is measured as physical work.

Summary

1 A significant reduction of the resting pulse rate occurred in the women in the weight reduction group

2. A significant decrease in the post exercise pulse rate was observed in both sexes in the weight reduction group

3. With respect to resting blood pressure, a tendency towards a decrease was present in all groups and there were no distinct differences between the groups.

4 No significant changes in resting ventilation were found in any group

5 A highly significant reduction in ventilation during exercise occurred in the weight reduction group a distinct difference being observed in comparison with the control groups. The reduction corresponded to the amount of weight loss as indicated by the significant correlation coefficient between reduction in ventilation during exercise and the relative weight loss.

6 The same observations were made with respect to oxygen consumption during exercise.

7 A low caloric diet in itself, without loss of weight, does not seem to produce any significant changes in the investigated functions.

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Fever of Obscure Origin

A Follow-up Investigation of 88 Cases

By

Tor Pettersson

Continuous fever of obscure origin is a not uncommon phenomenon in clinical practice, which often puts the diagnostic skill of the physician to hard tests. After many weeks of careful examinations we are sometimes forced to admit our failure in establishing a diagnosis. In order to facilitate the understanding of these obscure cases it seems wise to learn from the past and perform a follow-up investigation of patients discharged from hospital with the diagnosis of fever of obscure origin, here abbreviated to FOO

I previous follow-up investigations tuberculosis, malignant tumours and systemic diseases, collagen diseases and miscellaneous infections have been mentioned as the commonest late diagnoses made. In Scandinavia the problem which FOO presents has been discussed in 1955 in this journal by Böttiger (3) who reviewed the previous literature. In the present paper only a few studies will be briefly mentioned. Alt & Barker (1) published follow up study of 101 cases. In 23 per cent the diagnosis was established. There were 6 cases of tuberculosis, 6 cases of rheumatic fever and 6 cases of malignant tumour Kintner & Rowntree (9) who studied a series of 100 cases, pointed out that a diagnosis of tuberculosis had been erroneously made in many cases during some phase of the illness. Hamman & Wainwright (7) made a definite diagnosis in 34 cases out of 54 in which FOO had been present. Nine of these patients had tuberculosis, 10 had syphilis, 8 had malignant tumours and 5 had miscellaneous infections. These writers held that a late diagnosis can be made in about 40 per cent of cases. Böttiger himself (3) described a series of 158 cases from the 10-year period 1940—1949 In 35 cases, i. e. about 22 per cent, a diagnosis was later established, tuberculosis and malignant tumour being the dominant diseases. The series included 6 cases of tuberculosis, 11 cases of malignant tumour 4 cases of collagen disease and 11 cases of miscellaneous infections. Böttiger emphasized that the disease often

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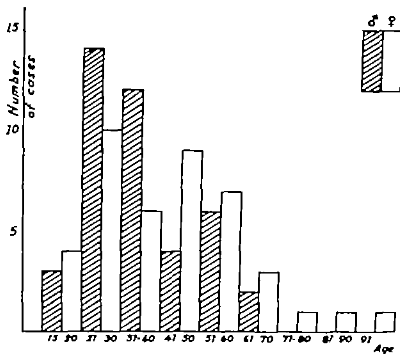


Fig 1 Distribution by age and sex.

was serious by nature, 19 patients had died Geraci et al. (6) pointed out that exploratory laparotomy in cases exhibiting FOO is useful Their series included 70 patients. In about 80 per cent of cases exploratory laparotomy provided a clue to the diagnosis, and in 60 per cent a definite diagnosis could be made. The majority of patients had malignant diseases e. g cancer of the pancreas or colon, or lymphoblastoma. For further literature the reader is referred to the monograph by Keefer & Leard (8)

The concept of FOO is rather indeterminate. This denomination is used when a febrile state persists for some length of time without any etiological cause being detected in spite of thorough examinations. But there is no clear consensus as to the duration and level of the fever Some writers (6) hold that hyperthermia has to be present for at least two weeks to justify the use of the term FOO Others (1 3) include in their series patients who have had fever of less than 10 days duration. Opinions also differ in regard to the distinction between the normal temperature and fever Many papers have been published on the normal body temperature, and the views are conflicting Kintner & Rowntree (9) for instance, held that a temperature of over 37 2 C should always be regarded as fever By contrast, according to Best & Taylor (2) the average temperature is as high as 37.5 C with variations of 0.5—1 C during the day Böttiger (3) emphasized that a sharp distinction cannot be drawn at 37 C, there being considerable variations within the normal range It is not my intention to discuss the normal body temperature and the factors influencing it, since papers are available on this subject to which the reader is referred (3 9 10 11 12)

Before one is justified in speaking of FOO the patient should be carefully examined. The examination should include a complete blood picture examinations of the urine and the faeces, blood cultures, electrophoresis and all the usual serological tests (the antistreptolysin and anti-

Table I

	No. of cases of FOO			No. of cases of FOO in which diagnosis was later made		
	Men	Women	Total	Men	Women	Total
Fever of 6—10 days' duration						
) ESR under 30 mm/hr.	10	7	17	1	1	2
b) ESR over 30 mm/hr.	4	7	11	—	2	
Fever of over 10 days duration						
a) ESR under 30 mm/hr.	8	7	15	1	4	5
b) ESR over 30 mm/hr	19	21	40	6	10	16
Total	41	42	83	8	17	25

staphylolysin titres Widal, Bang, Weil-Felix, a test for toxoplasmosis) The patient should also be examined for the presence of LE cells. Furthermore, a roentgenological examination of the lungs, gallbladder and kidneys, a gynaecological examination and a rectal examination should be made. In cases in which fever has been present for a relatively short period, for instance for 10 days, all these examinations are often not performed, as appears from several previous investigations.

Material and methods

The series consists of 88 patients hospitalized at the Aurora Hospital Medical Department for Adults, Helsingfors, during the 10-year period 1950—1959 and discharged with the diagnosis of FOO I order to eliminate common acute *infections*, certain neurovegetative disturbances, physiological premenstrual elevation of temperature and hyperthermia *in connection with thyrotoxicosis* only cases in which fever persisted for at least six days and sometimes exceeded 38 C were included.

All patients were requested to attend follow-up examination, which included detailed interrogation regarding the history of the disease blood studies, examination of the urine, the erythrocyte sedimentation rate and roentgenological examination of the lungs. Five patients could not be found and were therefore omitted. *Hence, the series proper* consists of 83 patients. Twelve patients, who did not come to follow-up examination, could be reached by mail or by a personal call. The annual frequency of FOO varied between 3 and 15 cases. The majority of patients were between 20 and 40 years of age, and there were 42 women and 41 men. The distribution by age and sex appears in fig I

In order to facilitate analysis of the material I have divided it into two main groups, using the same principles of classification as Böttiger (3) in order to enable comparison between the two studies. Group I consists of patients *with fever of 6—10 days' duration*, group II of patients with fever of over 10 days' duration. Both groups have been divided into two subgroups,) with a normal or slightly elevated ESR under 30 mm, and b) with a definitely elevated ESR over 30 mm. Table I shows the distribution of the patients by this classification.

As rule, those patients who had had fever of less than 10 days duration had not been subjected to all the examinations mentioned in the foregoing. In all cases roentgenological examination of the thorax and examinations of the urine and faeces and complete blood studies had been performed. The Widal test is lacking in only few cases. Vaginal and rectal examinations had been made in all cases. The Bang and Weil-Felix tests had been performed in 80 per cent of cases. In those cases in which fever had persisted for over *10 days*, examinations of the gallbladder and kidneys had mostly been performed, and the

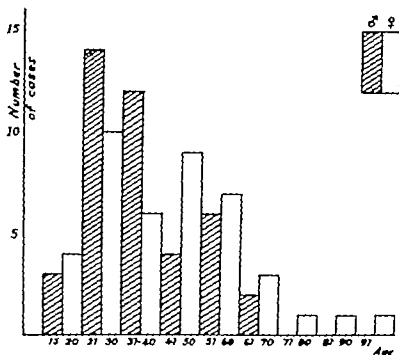


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Fever of over 10 days duration, ESR under 30 mm/hr

This group consists of 15 patients. The average hospital period was 20 days. In 5 cases the diagnosis could be established. One patient had died, and this case will be described briefly.

A 53-year-old man. Before admission he had had fever for eight weeks. Throughout the hospital period he had high fever (fig. 2). On admission ESR was 27 and 41 days later 2 mm/hr. Notwithstanding careful examination nothing noteworthy was detected. The heart was slightly enlarged, but no murmurs were heard. The patient was transferred to another hospital, exploratory laparotomy being meditated, but he died before this operation was performed. At autopsy verrucous endocarditis was detected.

One case of tuberculosis was detected in which a relationship with the period of hyperthermia seemed obvious. Collagen disease was observed in 2 cases. In one of them, periarteritis nodosa seems



Fig. 2.

to have been involved. Eosinophilia of 11.5 per cent was present. The patient has been persistently ill since discharge from the hospital, and there has been intermittent fever. Nephropathy with microscopical haematuria and hypertension have developed. The patient has had joint symptoms from time to time. Histologically however the diagnosis is not yet verified. In the other case typical chronic rheumatoid arthritis is involved, joint symptoms developed some months after the period of obscure fever.

This group includes a case of uterine carcinoma, which undoubtedly was connected with the febrile state. The patient was a 50-year-old woman, who was hospitalized for 22 days and had intermittent fever of 38° C. A gynaecological routine examination revealed nothing noteworthy. Six months later uterine carcinoma was detected. After operation the fever disappeared.

The 2 cases of simulation already mentioned belonged to this group. One of them seems worthy of a brief description.

A 41-year-old unmarried woman, who was admitted for FOO. Nothing pathological was observed notwithstanding careful examinations. Gradually the temperature was normalized and the patient was discharged. Four years later she was again admitted for FOO. The temperature showed marked variations during the day. Gradually the suspicion arose that simulation was involved, and check-up determinations confirmed this. It was found

Table II Results in the follow-up study

	No. of cases
Diagnosis established	25 (including 6 deaths)
Simulation	2
Cause remained obscure	56 (including 1 death)
Total	83

Table III Diagnosis later established in patients with FOO

	No. of cases	Total no. of cases	Deaths
Tuberculosis			
Tub. pulm.	7		
Pleuritis tub.	1	9	2
Spondylitis tub.	1		
Malignant tumours and systemic diseases			
Ca pulm.	1		
Ca mammae	1	5	3
Ca uteri	1		
Lymphogranulomatosis maligna	1		
Sarcoidosis	1		
Collagen diseases			
Rheumatoid arthritis	2		
Lupus erythematosus disseminatus	2	5	—
Periarteritis nodosa (?)	1		
Miscellaneous infections			
Pyelonephritis	2		
Cholecystitis	2	6	1
Abscess	1		
Endocarditis	1		
	Total	25	6

gastro-intestinal tract had been examined in many cases. The antistreptolysin titre had been determined in 50 per cent of cases, the antistaphylolysin titre in 25 per cent of cases. Blood cultures had been done in about 35 per cent of cases. During the last years covered by this study examinations for the presence of LE cells and electrophoretic studies had often been performed.

Results

As appears in table II a diagnosis which with all likelihood bears a relationship to the occurrence of FOO could be established in 25 cases, i. e. in about 30 per cent. Furthermore, 2 cases of simulation were detected. Table III shows the diagnoses made in these 25 cases. Six of these patients had died, and one patient had died from a disease which was not related to the period of hyperthermia.

Fever of 6—10 days' duration, ESR under 30 mm/hr

This group consists of 17 patients. They had been hospitalized for an average of 11 days. All were free from fever when discharged from the hospital. At follow up examination pulmonary tuberculosis was detected in 2 cases. One of these patients had been hospitalized on account of FOO one year before. At this time an X ray picture of the thorax revealed nothing pathological. Now active tuberculosis was present in the right lung. Löwenstein was positive. The other patient had been admitted for FOO 10 years ago. A roentgenological examination of the thorax showed nothing noteworthy but at follow up examination changes indicative of chronic tuberculosis were detected in the apex of the left lung. A relationship with the period of hyperthermia seems possible. The other patients were healthy.

Fever of 6—10 days' duration ESR over 30 mm/hr

This group consists of 11 patients. The average hospital period was 18 days. All patients were free from fever when discharged from the hospital. A 56-year old man had died from a heart disease which bore no relation to FOO. In 2

cases biliary disease was detected. In one of these, cholecystitis was observed shortly after the period of fever of unknown origin. The other patient had gall-stones, but during the hospital period she had no symptoms from the biliary tract. However soon after discharge she developed clear cholecystitis. One patient in this group, a 44-year-old man, showed eosinophilia of 16—29 per cent during the time of hospitalization. The leukocyte count varied between 7 700 and 5,500. ESR was about 80 mm/hr Periarteritis nodosa was suspected but no convincing evidence was obtainable. Since discharge from the hospital the patient has been completely healthy At follow up examination seven years later the blood picture, ESR and an X-ray picture of the thorax were completely normal.

Fever of over 10 days' duration ESR under 30 mm/hr

This group consists of 15 patients. The average hospital period was 20 days. In 5 cases the diagnosis could be established. One patient had died, and this case will be described briefly

A 53-year-old man. Before admission he had had fever for eight weeks. Throughout the hospital period he had high fever (fig 2) On admission ESR was 27 and 41 days later 2 mm/hr Notwithstanding careful examination nothing noteworthy was detected The heart was slightly enlarged, but no murmurs were heard. The patient was transferred to another hospital, exploratory laparotomy being meditated, but he died before this operation was performed. At autopsy verrucous endocarditis was detected.

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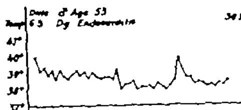


Fig. 2

to have been involved. Eosinophilia of 11,5 per cent was present. The patient has been persistently ill since discharge from the hospital, and there has been intermittent fever Nephropathy with microscopical haematuria and hypertension have developed. The patient has had joint symptoms from time to time Histologically however the diagnosis is not yet verified. In the other case typical chronic rheumatoid arthritis is involved joint symptoms developed some months after the period of obscure fever

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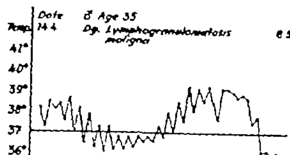


Fig 3.

that the patient had disease-insurances in four different insurance companies. She never admitted simulation but it was found that she had been caught for simulation one year before in another hospital, to which she had been admitted on account of a slight accident

One patient a 21 year-old man developed acute pericarditis four years after he had had FOO but there did not seem to be any relationship between the two conditions. Another patient had developed malignant hypertension The other patients of this group were healthy

Fever of over 10 days' duration, ESR over 30 mm/hr

This is the largest group comprising 40 patients. The average hospital period was 39 days In this group a diagnosis could be established in 16 cases, i. e. in 40 per cent of cases. Five patients had died. There were 6 cases of tuberculosis. In 5 of these the tuberculous process was observed within one year after the period of fever In the sixth case the interval was five years, and in this the relationship cannot be regarded as established. In 3 cases pulmonary tuberculosis was involved. In one of these there was also tuberculosis of the bone. One patient had tuberculous spondylitis Two of the patients with tuberculosis had died. There were 2 cases in which lupus erythematosus disseminatus was detected. Both these patients were women and LE cells were later repeatedly observed in the blood in both One of these cases may be described.

A 51 year-old woman. Before admission there had been fever of about 38 C for some length of time and occasional joint symptoms. The patient was hospitalized for 83 days without any diagnosis being established. On admission signs indicative of pleuritis to the left were observed. Repeated tests for LE cells were negative and blood cultures gave no results. Careful roentgenological and laboratory examinations gave negative results. Finally exploratory laparotomy was performed though without enabling a diagnosis to be made. ESR was consistently high, varying between 70 and 105 mm/hr Antibiotic therapy was given several times without influencing the temperature. Finally cortisone treatment was given with a favourable result. At this time sarcoidosis was suspected. One year later the patient was again admitted for fever She had then been without cortisone for some length of time. Now several tests revealed LE cells in the blood and the diagnosis of lupus erythematosus disseminatus could be established

The group under discussion includes one case of malignant lymphogranulomatosis which will be briefly described.

A 35-year-old man, who before admission had had bouts of fever for about four months, each time lasting about a week. He was admitted to the hospital during such a period. The general condition was good ESR was 58 mm/hr an X-ray picture of the thorax showed nothing noteworthy the blood picture was normal, Widal, Bang and Weil-Felix were negative No lymph nodes were palpable The patient had fever of the Pel Ebstein type (fig 3) Since at this time there had been several cases of bovine febris undulans in the patient's domicile this disease was strongly suspected but no diagnosis had been made when the patient was discharged. ESR had dropped to 22 and the temperature was normal. For about six weeks the patient was in good condition at home but then fever returned. The patient was readmitted. Now there were palpable lymph nodes. A histo-

logical examination showed Reed-Sternberg's giant cells. The diagnosis of malignant lymphogranulomatosis was made and verified at autopsy two years later.

The following case of sarcoidosis occurred in this group of patients.

A 37-year-old woman, who had had persistent fever of about 38° C for some three months before admission. She had repeatedly received antibiotic therapy without any effect. On admission the patient was in rather poor condition, she had lost weight. There was slight hypochromic anaemia. Several roentgenological examinations of the thorax failed to reveal anything pathological, and all other tests were likewise negative. Throughout the hospital period the temperature was continuously about 38° C (fig. 4). ESR varied between 62 and 22 mm/hr. The patient was discharged from the hospital after 47 days, when the temperature had been about 37.5° for some days. At home she again had persistent fever of about 38°. She was readmitted ten months later after having had fever all the time. Now some cervical lymph nodes were palpable. After histological examination the diagnosis of sarcoidosis could be established. The lymph node consisted of 90 per cent of epitheloid and giant cells. Furthermore, an X-ray picture of the thorax revealed enlarged hilar shadows. The patient was given cortisone and is now in fairly good condition.

Two cases of chronic rheumatoid arthritis and 3 cases of unspecific infection were encountered. Among the latter there were 2 cases of pyelonephritis. One of these patients has died from the disease. The other case may be briefly described.

A 60-year-old man, who had received antibiotic therapy prior to admission. He had then had fever for two weeks. On admission the catheter urine was normal, and urography revealed nothing pathological. Other examinations gave negative results. The fever disappeared and the patient was discharged after 27 days hospitalization. Soon afterwards he again developed fever and this

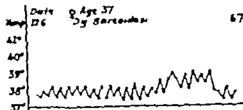


Fig. 4

time also difficulty in urination. The patient was readmitted, and it was soon established that he had chronic pyelonephritis, which had escaped recognition the first time owing to the antibiotic therapy given.

Furthermore, there were 2 cases of malignant tumour. In one of them cancer of the lung was involved, and this patient has died. The other patient had metastasizing breast cancer and she too has died.

Discussion

Just as in many previous studies (1, 3, 7) tuberculosis was one of the dominant diseases encountered in this follow-up investigation. A total of 9 patients, i. e. one-tenth of the whole series, had this disease. This is more than in Böttiger's Swedish series (3) which seems natural considering that tuberculosis is more frequent in Finland than in Sweden. The majority of these cases belonged to group II.

The incidence of collagen diseases was also relatively high, which tallies with the results of others (3). Among the 5 cases diagnosed there were 2 cases of lupus erythematosus disseminatus, a disease which has not always been encountered by previous writers. This is probably due to the fact that the possibilities of diagnosing it have much improved during the last ten years, and therefore greater attention is now directed to its occurrence.

The incidence of malignant tumours and systemic diseases was about the same as that of collagen diseases. There was no case of hypernephroma in this group but in the presence of protracted fever of unknown origin this form of tumour should always be suspected (4 5 13). It is striking that no case was encountered in which the febrile state could be attributed to a malignant process in the viscera. The shortest time of observation one year ought to be sufficient for a malignant process to develop. Geraci et al. (6) reported that the performance of exploratory laparotomy enabled them to make a diagnosis in 60 per cent of cases in their series of patients with FOO. When my results are analysed it appears that in the present series exploratory laparotomy would not have been equally helpful an aid.

Miscellaneous infections also constitute an important group. The case of pyelonephritis described shows that one test taken immediately after admission to hospital is not enough, the examinations should be repeated. Antibiotic drugs are today used to such an extent that the majority of patients have received such treatment one or more times prior to admission. Often the treatment has not been effective enough to cure the patient, the only result being a temporary improvement and transient normalization of significant laboratory tests.

In many cases of FOO common diseases are involved which occur in an atypical form and therefore escape recognition. The problem which FOO presents is always interesting and puts the diagnostic skill of the physician to hard tests. Experience has shown that the patient should be kept under observation for a considerable length of time even after he [illegible] have recovered. In over one fourth of cases a diagnosis can be made later. Particular attention should be paid to the possible presence of tuberculosis, malignant tumours, systemic diseases and collagen diseases.

However a large proportion of cases remain obscure, and the majority of these patients recover completely. It seems possible that many of them suffered from infectious diseases caused by bacteria, viruses or other microorganisms, which in spite of thorough examinations escaped recognition. A tuberculous infection is not always accompanied by demonstrable changes of the lungs or other organs. Thanks to the tomographic method the chances of establishing an early diagnosis have improved, however. Considering that the present series includes 9 cases of tuberculosis it seems possible that some of the patients who had recovered had had a tuberculous infection which had caused no demonstrable changes. Furthermore, it is a well-known fact that typhoid and paratyphoid infections do not invariably cause elevated Widal titres, and sometimes bacteria are not found in the faeces or the urine. It should also be borne in mind that the results of examinations of the faeces and urine may be influenced by repeated antibiotic treatment given prior to admission to hospital and the performance of systematic examinations.

Diseases caused by microorganisms which are familiar to us may, then, remain undiagnosed when cultures and serological findings are negative. But it should also be borne in mind that protracted fevers undoubtedly exist which are due to bacteria, viruses or other microorganisms not yet recognized.

Systemic diseases and systemic reactions may also run a course without the development of changes enabling the

establishment of a diagnosis. Protracted febrile states may also be due to systemic reactions which are not yet understood.

Summary

A follow-up study was performed on a series of 88 patients discharged from hospital with the diagnosis of fever of obscure origin (FOO). Eighty-three patients could be reached, i. e. 41 men and 42 women. The majority were aged 20—40 years.

A diagnosis related to the febrile state could be established in 25 cases, i. e. in about 30 per cent. There were 9 cases of tuberculosis, 5 cases of malignant tumour or systemic disease, 5 cases of collagen disease and 6 cases of miscellaneous infections. Of these patients 6 had died, namely 2 of tuberculosis, 2 of malignant tumours, one of malignant lymphogranulomatosis and one of endocarditis.

In 56 cases the diagnosis remained obscure. The majority of these patients were found to be in good health. The possible sources of the disease are discussed, and it is emphasized that in many cases it was probably caused by familiar bacteria, viruses or other microorganisms, which had escaped recognition owing to the absence of demonstrable changes of the tissues and serological reactions. It seems probable that tuberculosis was involved in some cases in this group. There is no doubt, however that pyrexial diseases also exist which are due to microorganisms that have not yet been identified. Furthermore, there are systemic diseases and systemic reactions known to run a similar course without demonstrable changes and ending in recovery but the source of the febrile state may also be a systemic reaction not yet understood.

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Miscellaneous infections also constitute an important group. The case of pyelonephritis described shows that one test taken immediately after admission to hospital is not enough, the examinations should be repeated. Antibiotic drugs are today used to such an extent that the majority of patients have received such treatment one or more times prior to admission. Often the treatment has not been effective enough to cure the patient, the only result being a temporary improvement and transient normalization of significant laboratory tests.

In many cases of FOO common diseases are involved, which occur in an atypical form and therefore escape recognition. The problem which FOO presents is always interesting and puts the diagnostic skill of the physician to hard tests. Experience has shown that the patient should be kept under observation for a considerable length of time even after he seems to have recovered. In over one-fourth of cases a diagnosis can be made later. Particular attention should be paid to the possible presence of tuberculosis, malignant tumours, systemic diseases and collagen diseases.

However, a large proportion of cases remain obscure, and the majority of these patients recover completely. It seems possible that many of them suffered from infectious diseases caused by bacteria, viruses or other microorganisms, which in spite of thorough examinations escaped recognition. A tuberculous infection is not always accompanied by demonstrable changes of the lungs or other organs. Thanks to the tomographic method the chances of establishing an early diagnosis have improved, however. Considering that the present series includes 9 cases of tuberculosis it seems possible that some of the patients who had recovered had had a tuberculous infection which had caused no demonstrable changes. Furthermore, it is a well-known fact that typhoid and paratyphoid infections do not invariably cause elevated Widal titres, and sometimes bacteria are not found in the faeces or the urine. It should also be borne in mind that the results of examinations of the faeces and urine may be influenced by repeated antibiotic treatment even prior to admission to hospital and the performance of such examinations.

Diseases caused by microorganisms which are familiar to us may thus remain undiagnosed when cultures and serological findings are negative. But it should also be borne in mind that protracted fevers undoubtedly exist which are due to bacteria, viruses or other microorganisms not yet recognized.

Systemic diseases and systemic reactions may also run a course without the development of changes enabling the

establishment of a diagnosis. Protracted febrile states may also be due to systemic reactions which are not yet understood.

Summary

A follow-up study was performed on a series of 88 patients discharged from hospital with the diagnosis of fever of obscure origin (FOO). Eighty-three patients could be reached, i. e. 41 men and 42 women. The majority were aged 20—40 years.

A diagnosis related to the febrile state could be established in 25 cases, i. e. in about 30 per cent. There were 9 cases of tuberculosis, 5 cases of malignant tumour or systemic disease, 5 cases of collagen disease and 6 cases of miscellaneous infections. Of these *patients* 6 had died, namely 2 of tuberculosis, 2 of malignant tumours, one of malignant lymphogranulomatosis and one of endocarditis.

In 56 cases the diagnosis remained obscure. The majority of these patients were found to be in good health. The possible sources of the disease are discussed, and it is emphasized that in many cases it was probably caused by familiar bacteria, viruses or other microorganisms, which had escaped recognition owing to the absence of demonstrable changes of the tissues and serological reactions. It seems probable that tuberculosis was involved in some cases in this group. There is no doubt, however that pyrexial diseases also exist which are due to microorganisms that have not yet been identified. Furthermore, there are systemic diseases and systemic reactions known to run a similar course without demonstrable changes and ending in recovery but the source of the febrile state may also be a systemic reaction not yet understood.

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Acute Muscular Syndrome in Chronic Alcoholism

By

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In veterinary medicine acute degenerative or necrotic muscle changes of non-neurogenic type may be met with in different pathologic conditions of widely varying etiology and pathogenesis. In animals degeneration and necrosis of muscle can also be induced experimentally in several different ways (1, 2, 3, 4).

In the field of human medicine, muscle changes in cases of exogenic intoxication are little known. Myopathy has been described in certain cases of carbon monoxide poisoning, however (5, 6, 7). Haff disease is also thought to be caused by an exogenic intoxication (8), and during recent years muscular lesions caused by sea snake poisoning have been reported (9).

In 1955 (10) and 1957 (11) we published preliminary reports on a symptom complex in chronic alcoholism not previously described. This is characterized primarily by aching, tenderness and edema in the musculature, accompanied in the majority of cases by renal damage with hyperpotassemia. Histologic examination showed muscle damage with necrosis. All patients were chronic alcoholics, and the muscular lesions were noted in association with periods of immoderate alcohol consumption.

We have been unable to find any reports in the literature of similar cases. As we have had an opportunity to study an additional seven cases presenting similar symptoms, a more detailed description of the symptoms accompanying the changes in question and a more complete presentation of the cases seems justified.

Material and methods

The material consists of a series of 12 cases (10 men and 2 women) aged from 41 to 58 years (table I). All patients had a history of many years of high-grad abuse of alcohol and all except one (case 8) had been under the supervision of the National Temperance Committee. Seven patients had received treatment for alcohol addiction in institutions for treatment of alcoholism or mental hospitals. Four of the patients died, three of them (cases 1, 4 and 7) in conjunction with acute muscular necrosis and one (case 9) a few weeks after discharge in delirium tremens.

Methods. The GOT activity was determined according to Karmen's (12) method, modified by Ordell (13). Normal values 10—25 units.

The GPT activity was determined according to modified La Due, Wróblewski, Kar

After completion of the manuscript two reports of similar cases have appeared in the literature (37, 38).

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Table I Case material

Case no.	Sex and age	Edema, localization	Comments
1	M 48	Right upper arm and shoulder	Died of renal failure with hyper kalemia
2	M 45	Left thigh	Myoglobinuria
3	M 44	Both gluteal regions and calves and left thigh	—
4	M 53	Both calves	Myoglobinuria. Died of renal failure with hyper kalemia
5	M 42	No edema	Myoglobinuria. Renal failure
6	M 52	Left thigh and gluteal region	Renal failure with hyperkalemia. Polyneuritis in both hands
7	F 58	Both arms	Died of bilateral bronchial pneumonia
8	M 51	No edema	—
9	M 49	No edema	Died of delirium tremens. No autopsy
10	M 48	Left calf	Polyneuritis in both hands
11	M 41	Both gluteal regions and right thigh	Left-sided radial paresis. Myoglobinuria
12	F 44	Right thigh	Renal failure

men (14) technique. Normal values 8—22 units.

The LD activity was determined according to Ordell (15) normal values 10—25 units.

The aldolase activity was determined according to Sibley and Lehninger (16) Normal values 3—10 units.

Muscle biopsy specimens were excised under local anesthesia of the cutaneous and subcutaneous tissues. The histologic material was fixed in Bouin's solution embedded in paraffin and sliced. Hematoxylin-eosin and van Gieson staining were used.

Liver biopsy specimens were obtained with a Vim-Silvermann needle.

Case reports

Cases 1 to *5* have been reported earlier the reader is referred to Acta med. scand. 1955 (11) and 1957 (12)

Case 6. The patient was a 52 year-old male construction worker who had for many years been strongly addicted to alcohol. He had a history of 26 arrests for intoxication. Jan. 2, 1959 the patient woke up in the morning with aching and tenderness in the left thigh and gluteal musculature During the period shortly before admission he had consumed more than 1/2 liter of distilled spirits daily as well as a number of bottles of beer The patient called a physician, who found Lasègue's sign positive, 45 and referred the patient to the hospital under the diagnosis of lumbago-ischias.

On admission to the hospital the patient was not intoxicated but was barely able to stand because of pain and tenderness in the left leg He had pronounced edema in the left thigh and gluteal region (fig 1) Reflexes and sensibility were normal, but he exhibited signs of polyneuritis in both hands.

At the time of admission the patient had symptoms of renal damage with secretion of small quantities of urine during the first week, varying between 100 and 250 ml/24 hours. No myoglobin was demonstrable spectroscopically in the urine. The non-protein nitrogen was considerably increased, with a maximum of 240 mg/100 ml and hyperpotassemia was demonstrated with 8.4 mEq the highest level. Electrocardiographic changes with high, pointed T waves accompanied the hyperpotassemia. After a week of conservative treatment urine was again secreted in normal amounts, and after four weeks the non-protein nitrogen was normal. Two and a half months after admission the patient had normal renal function as demonstrated by creatinine clearance and concentration tests.

The activities in serum of liver and muscle enzyme are shown in fig 3 Examination of liver biopsy material two months after the acute stage showed a normal histologic picture

Histologic examination of muscle biopsy material from the left gluteal musculature (clinical symptoms) (fig 7) showed the transverse striation to be well preserved on the

whole. As a rule the muscle fibers were of ordinary thickness. In some areas isolated or small groups of muscle fibers presented marked histologic changes. These were characterized primarily by more or less complete necrosis, at times with granular disintegration of the sarcoplasm. Other muscle fibers were hyalinized and showed a structure most nearly resembling Zenker degeneration. Here and there giant cell formation was seen. There was sparse leukocyt infiltration and an occasional myocyte. No definite regeneration was seen.

Biopsy specimens from the right quadriceps (clinically without symptoms) showed no histologic changes.

After three months the patient had recovered and was discharged from the hospital.

Case 7 The patient, woman aged 58 years, had for many years consumed large quantities of alcohol daily in the form of both distilled spirits and ine In 1956 when she was treated for bleeding gastric ulcer an enlarged liver extending two fingerbreadths below the arcus costarum was demonstrated. Dec. 15, 1958 the patient comparatively suddenly felt intense aching and tenderness in the musculature of the extremities. The symptoms increased in severity until the patient became completely incapacitated. She was admitted to the hospital Dec. 22, 1958.

On admission she had considerable edema in both arms (fig. 2) and intense tenderness in both arm and leg musculature. Reflexes and sensibility were normal. It could not be determined if there was muscular weakness because of the intense pain and tenderness.

The liver extended four fingerbreadths below the arcus. Nothing else noteworthy was pparent from clinical examination.

The activities of serum enzymes are shown in fig. 4

No myoglobin was demonstrable spectroscopically in the urine. The non-protein nitrogen was 41 mg/100 ml

Biopsy material from the left biceps brachii (fig. 8) revealed in general only slight atrophy with somewhat thin muscle fibers and an increase in the number of sarcolemma nuclei. The striation of the muscle fibers was clearly apparent in these parts. Pronounced changes were present, however in several small areas. Groups of muscle fibers there showed complete necrosis with loss of cross-striation, swelling and disintegration, karyorrhexis and karyolysis. In these regions marked edema was present interstitially with groups of inflammatory cells here and there, mainly neutrophil leukocytes. N vascular changes were observed and no signs of regeneration.

The patient's condition seemed to improve but after three weeks signs of bilateral bronchial pneumonia ppeared and the patient died despite massive antibiotic therapy

Autopsy showed an extremely advanced respiratory tract infection with purulent tracheobronchitis and with widespread, confluent bronchopneumonia with suppuration pervading both lungs. No changes of impor tance were found in the heart and vessels nor in the central nervous system. The liver was enlarged nd of firm, almost hard consistency the cross section showed indication of incipient cirrhosis. Macroscopic inspection of the kid ney and other internal organs revealed no pathological changes of importance. The skeletal musculature was generally notably flaccid and flabby with wet, greyish-yellow streaky cross-sections. There were no noteworthy dermal changes

The *histologic examination* showed no definite pathologic changes in numerous sections prepared from different parts of the brain, spinal cord and peripheral nerves. Nor were any notable changes demonstrated in the myocardium, spleen, adrenal glands or pituitary Slides from the liver showed pronounced degeneration of liver parenchymal cells with large fat globules. The normal tissue structure was obliterated and replaced by pseudo-acini of somewhat varied size There was a rather marked increase of the periportal connective tissue Accordingly this was case of pronounced fatty degeneration of the liver with relatively advanced periportal cirrhosis (fig 6).

The kidneys showed no noteworthy glomerular changes The tubuli were generally dilated with low epithelium. In several places tubuli were seen to contain compact cylinders of strongly acidophilic material of undefinable nature (fig 11)

At autopsy specimens taken from a number of differen muscle groups all showed essentially the same changes. The changes were extremely grave in the pharyngeal musculature, the sternocleidomastoid muscle, the pectoralis major the iliopsoas and the quadriceps femoris (fig. 9) but less severe in the

biceps brachii and the diaphragm. Histologically the picture was dominated by necrosis of the muscle fibers, varying from swelling and indistinct striation to complete coagulation, necrosis and disintegration with karyorrhexis and karyolysis. In the less affected regions the muscle fibers generally showed more or less marked atrophy with scattered smaller areas of necrosis. Where the changes were most advanced large, almost structureless areas, consisting of a granular mass of pleriophilic material without signs of nuclear residues, were seen as well as regions with well-preserved sarcolemma and densely distributed sarcolemma nuclei but with completely destroyed muscle fibers. Perivascularly and in connection with the necrotic regions a few inflammatory cells were seen, in some areas preponderantly lymphocytes and macrophages, in others mainly neutrophil leukocytes. In addition there was pronounced interstitial edema and usually slight or moderate fatty infiltration and increased interstitial connective tissue. Nowhere were signs of regeneration seen and no vascular changes were observed.

Case 8 The patient, a 51 year-old business man, had for many years consumed more than 1/2 liter of distilled spirits daily Psychic stress the last two months before his admission to the hospital further accentuated the alcohol abuse. Jan. 20, 1959 the patient had a sudden attack of severe aching and tenderness on the outer side of the left thigh. Even the pressure of the bedclothes caused intense pain. As he was unable to rise from the bed or to stand on the leg without assistance, he called a physician, who referred him to the hospital.

On admission the reflexes and sensibility were normal, but the patient had extreme tenderness on the outer aspect of the left thigh. No edema was palpable however Physical examination revealed nothing else pathologic.

The activity of liver muscle enzymes is shown in table II. The non-protein nitrogen was 40 mg/100 ml. Liver biopsy showed fatty degeneration.

Muscle biopsy from the left thigh (fig 10) revealed very limited pathologic changes. Thus in isolated muscle fibers small segments could be seen where the fibers were swollen, partly necrotic and had deformed structure. At these sites there was a complete loss of cross-striation. Slight disintegration of the sarcoplasm was observed. No infiltration of inflammatory cells or signs of regeneration could be seen.

After one month's hospitalization the patient was discharged wholly free from symptoms.

Case 9 The patient, a laborer aged 49 years, had a history of several admissions to institutions for treatment of high-grade alcoholism. For the past two years he had repeatedly suffered from intense aching and tenderness in calf musculature of both legs and, occasionally in the arms as well. During these periods he was completely incapacitated and forced to remain in bed. These symptoms occurred only after about a week's increased alcohol consumption however and never during the periods when he remained sober He was referred to the hospital for investigation of these symptoms.

On admission the patient complained of severe tenderness in arm and leg musculature but no edema was palpable. Reflexes and sensibility were normal. No paresis was demonstrable. The non protein nitrogen was 35 mg/100 ml.

The activities of serum enzymes are shown in table II Liver biopsy specimens revealed fatty degeneration.

The creatine excretion in the urine was 490 mg/24 hours.

Examination of biopsy specimens from the right gastrocnemius three weeks after the acute stage showed the tissue structure to be mainly normal. The muscle fibers varied little in thickness and exhibited distinct striation. Abundant sarcolemma nuclei were present in some areas where the sarcolemma cells also showed a certain degree of swelling with, in isolated areas, small nuclear conglomerates. Occasional muscle fibers were extremely narrowed and presented a great abundance of cell nuclei. The striation in such fibers was distinct. There was no accumulation of inflammatory cells. Vessels and nerves in the interstitial tissue showed nothing pathologic

Case 10 Th patient, a 48-year-old laborer had repeatedly received institutional treatment for alcoholism. After a period of extreme alcohol abuse he had a sudden attack of aching swelling and tenderness in the left calf The pain and tenderness were so severe

that he was unable to walk and was forced to remain in bed. He was admitted for investigation three weeks after the acute attack.

On admission the patient was almost free from symptoms. The left calf was still somewhat thicker than the right, but no muscular tenderness remained. Reflexes and sensibility were normal in both legs. Signs of polyneuritis were present in the hands.

Liver biopsy revealed fatty degeneration.

Muscle biopsy from the left gastrocnemius three weeks after the acute stage showed that the majority of the fibers were of ordinary thickness although some were narrowed to a greater or lesser degree. The sarcolemma cells were frequently swollen, and sarcolemma nuclei occurred abundantly in spots. Here and there thin, almost destroyed muscle fibers were seen, occasionally with tortuous course. The striation was distinctly visible in all areas in polarized light, and no signs of necrosis were observed. Small nuclear conglomerates were present in spots. Rather abundant histiocytes occurred in association with occasional minor blood vessels. Otherwise, there was no accumulation of inflammatory cells. At single muscle fiber was conglomerate of polygonal cells with intensely chromophilic cytoplasm and rounded or oval nuclei. These cells most nearly resembled myocytes.

Case 11 The patient, 41-year-old salesman, had been treated institutionally on several occasions for alcoholism. After period of extreme alcohol abuse the patient woke up one morning with aching and tenderness in both gluteal regions and thighs. He had difficulty in walking because of the muscular symptoms

On admission to the hospital the patient presented considerable edema in both gluteal regions and the right thigh. He also had left sided radial paresis although the nerve status was otherwise normal.

Liver biopsy showed fatty degeneration. The maximal non-protein nitrogen observed was 48 mg/100 ml. Myoglobin was demonstrable spectroscopically in the urine

No definitely pathologic structures were observed in biopsy specimens from the right gluteal musculature. The excised tissue included isolated minor nerves with ordinary appearance.

Serum enzyme activities are shown in table II and fig. 5.

The patient was discharged practically symptom-free after three weeks.

Case 12 The patient, a 44-year-old female worker had for many years been strongly addicted to alcohol. She had during the last five years several times been hospitalized for alcohol abuse. During the last week before admission she had consumed large quantities of alcohol.

On admission she was not intoxicated but was unable to stand because of pain and tenderness in the right thigh. Reflexes and sensibility were normal but she had pronounced edema in the right thigh.

During the first week after admission she secreted only small quantities of urine, varying between 50—200 ml/24 hours. The non-protein nitrogen was increased with a maximum of 225 mg/100 ml. Two months after admission the patient had normal renal function demonstrated by creatinine clearance and concentration tests.

No myoglobin was demonstrable spectroscopically in the urine.

The activities of serum enzymes are shown in table II

Histologic examination of muscle biopsy material from the right thigh showed more or less complete necrosis of segments of single muscle fibers. These segments were very thin and showed sometimes a finely granular disintegration of the sarcoplasm.

Around them many swollen sarcolemma nuclei were observed. No inflammatory cells were found.

Results

Subjective symptoms

The subjective symptoms consisted of muscular aching and tenderness. The pain was frequently extremely intense and the tenderness in the musculature so pronounced that the patients could scarcely touch the affected muscle regions. The symptoms were either localized to single muscles and muscle groups (cases 1 2, 6 8, 10 11 and 12) or more generalized (cases 3 4 5 7 and 9) In the most advanced cases practically all skeletal musculature was engaged. As a rule, the onset of the symptoms was

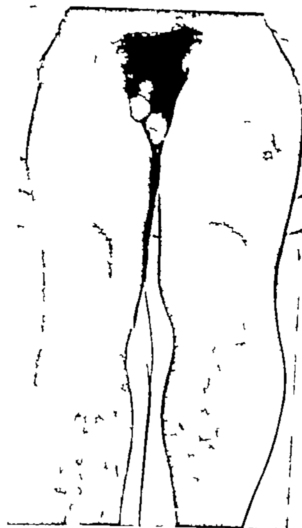


Fig 1 Case 6. Considerable swelling of the left thigh showing localization of the muscular edema.



Fig 2. Case 7 Considerable swelling of both arms showing localization of the muscular edema.

rather sudden and always (except in case 2) in connection with alcohol consumption. In case 2 the symptoms appeared in conjunction with acute barbituric acid poisoning while the patient was receiving treatment for alcoholism in a mental hospital Three patients (cases 4 5 and 9) had suffered repeated attacks of the aforementioned type, while the rest had undergone only one.

Local muscular status

In nine cases (1 2, 3 4 6 7 10 11 and 12) edema with some subcutaneous extension was palpated in the muscle regions involved. In several cases the symptom picture resembled that of deep-vein thrombosis. In case 4 the patient had had pain swelling and tenderness in the calves on several occasions wherefore the symptom picture was interpreted as thrombosis affecting the veins of the calf musculature. Repeated phlebograms showed normal conditions, however At autopsy after the patient died in conjunction with a new attack, no thrombosis was detected but extensive necrotic changes were found in the calf musculature. Case 10 also presented a symptom picture resembling thrombosis in one calf In case 1 where the symptom picture resembled that in axillary vein thrombosis, autopsy revealed no signs of thrombosis. Cases 2 and 6 presented swelling of one thigh and a condition similar to the clinical picture in femoral vein thrombosis (fig 1)

In the remaining four cases the appearance of the edema did not give rise to suspicion of thrombosis. Case 3 presented edema in both gluteal regions and the calves as well as the left thigh In

case 7 there were extensive changes with edema primarily in both arms (fig 2) Case 11 presented edema in the left gluteal region and thigh and case 12 only in the left thigh

In only three cases (5, 8 and 9) was no edema demonstrable by palpation.

Neurologic investigation

Signs of neuritis were present concurrently with the muscle symptoms in three cases (6, 10 and 11) Lasègue's sign was positive in three cases (5 6 and 8) In all the rest of the cases neurologic examination showed normal conditions.

Heart and circulation

Neither subjective nor objective signs of cardiac impairment were present in any case. Electrocardiographic examination showed a normal condition except in three cases where the patients exhibited ECG changes indicating hyperpotassemia. During the acute stage tachycardia occurred with frequencies up to 120/min in the majority of patients. As a rule, however the heart rhythm returned to normal within 14 days after admission.

Urine and renal function

Proteinuria was demonstrated during the acute stage in all cases. It persisted for only a few days or up to one week, however and in no case exceeded 0.2 %. Microscopic examination of urine sediment revealed only isolated or up to at most 10 erythrocytes per field.

Five cases (1 4 5, 6 and 12) presented renal damage with anuria-oliguria and a non-protein nitrogen elevation to a maximum of 191 56, 180, 240 and 225 mg/100 ml respectively Two of these patients (cases 1 and 4) died suddenly with signs of hyperpotassemia. The three patients with oliguria and elevated non-protein nitrogen levels who survived the acute stage had completely normal renal function according to results of creatinine clearance and concentration tests three months after the onset of symptoms. In five of the remaining cases (2, 3 7 8 and 11) the non-protein nitrogen ranged from 40 to 50 mg/100 ml, while in the other two it was below 40 mg/100 ml.

In four cases (2 4 5 and 11) the presence of myoglobin in the urine was verified spectrographically

Creatinuria

The creatine excretion in the urine was determined in four cases (2 8, 9 and 12) in the acute stage and found to range from 420 to 790 mg/24 hours. In three other cases (5 6 and 10) the excretion was determined only after the muscular symptoms had subsided. None of these patients had creatine excretion exceeding 50 mg/24 hours.

Blood and blood electrolytes

The hemoglobin concentration and the erythrocyte count were normal in all except one case (2) There was a rise in the hemoglobin value to 131 per cent and in the erythrocyte count to 6.1 million during the acute stage in this case. The blood values returned to normal within three days.

Of the blood electrolytes, potassium was elevated during the acute anuria-oliguria stage in three cases (1 4 and 6) to 7.0 12.4 and 8.4 mEq respectively In all three of these cases electrocardiographic examination also showed signs of hyperpotassemia, with high pointed T waves and in one case interventricular heart block as well. Two of these three patients (cases 1 and 4) died suddenly In cases 5 and 12, where the patients had oliguria and the non-protein nitrogen

Table II Results for serum enzyme activity (GOT GPT LD and aldolase) and histological diagnosis at liver biopsy Only the highest values for the respective enzymes are included

Case	Enzyme activity units				Liver biopsy
	GOT	GPT	LD	Al-dolase	
1	—	—	—	—	Fatty liver
2	280	—	—	—	Normal
3	380	—	144	—	Normal
4	—	—	—	—	Fatty liver
5	—	—	—	—	Portal cirrhosis
6	396	262	199	14 7	Normal
7	376	100	102	44	Portal cirrhosis
8	59	74	27	15	F tty liver
9	97	53	29	15	Fatty liver
10	14	10	20	8.5	Fatty liver
11	354	280	115	28	Fatty liver
12	232	180	60	34 6	Fatty liver

was elevated to 180 and 225 mg/100 ml respectively potassium was not determined. In the rest of the cases the serum potassium content was normal

Liver muscle enzymes in serum

In the last seven cases examined (cases 6—12) the GOT GPT LD and aldolase activities in serum were followed during the entire course of the disease in repeated determinations. In the five earlier cases (1—5) however only isolated determinations were made (table II) In six cases (2 3 6 7 11 and 12) the examination was carried out during the acute stage, when the muscular symptoms were most pronounced. In all these six cases there was an appreciable increase in the activity of the different liver muscle enzymes in the serum. In these six cases the GOT activity ranged from 280 to 396 units, GPT from 100 to 280 LD from 63 to 199 and aldolase from 15 to 44 units.

In three cases (8, 9 and 10) on the other hand, the activity of the different liver muscle enzymes in serum was not determined until the acute muscular symptoms had subsided. In case 8 determinations were done 10 days after the acute attack, in cases 9 and 10 three weeks after The increases in enzyme activity were considerably less marked in these cases, and in case 10 the activities of all liver muscle enzymes investigated were normal (table II)

It is evident from the cases in which the enzyme activity in serum was followed in repeated determinations that the time required for normalization of the enzyme activity can vary widely (fig 3—5)

Liver biopsy

Histologic examination of liver tissue was carried out in all cases (table II) on biopsy material in nine cases and autopsy material in three. Two patients (cases 5 and 7) had portal cirrhosis (fig 6) and three (cases 1 4 and 9) had advanced fatty degeneration of the liver while four (cases 8 10 11 and 12) had only moderate fatty degeneration In three cases (2 3 and 6) the liver presented a normal histologic picture. In these three cases, however the liver biopsy was not done until one or two months after the patients had been admitted to the hospital

Histologic examination of muscle tissue

In the three autopsy cases (1 4 and 7) extremely extensive and advanced histologic changes were demonstrated in the musculature (fig 9) These varied in intensity in different parts of the musculature and showed different degrees of degenerative changes, ranging from Zenker s degeneration to total destruction of

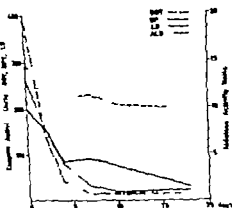


Fig. 3. Case 6. Repeated determination of enzyme activities.

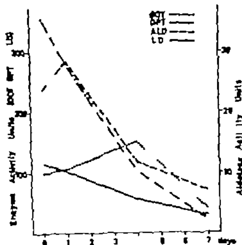


Fig. 5. Case 11. Repeated determination of enzyme activities.

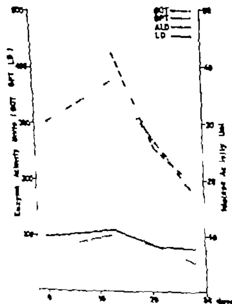


Fig. 4. Case 7. Repeated determination of enzyme activities.

muscle fibers. A prominent feature in all three cases was an extremely marked interstitial edema. Otherwise the picture was dominated by necrosis, at times very extensive.

The muscle biopsy specimens from a series of five cases (2, 6, 7, 8 and 12) presented degeneration and necrosis (fig. 7, 8 and 10) particularly in certain areas of the excised musculature and widely varying in degree in the different cases. Zenker's degeneration of isolated muscle fibers was common to the five cases. In the same biopsy specimens there was also granular disintegration of the sarcoplasm, empty sarcolemma tubes as residues after destroyed muscle fibers and, in two cases, marked extensive edema and, respectively, nuclear conglomerates and swelling of the sarcolemma cells. In cases 8 and 12 the pathologic changes were very limited, consisting only of degeneration and necrosis of isolated muscle fibers.

In case 3 empty sarcolemma tubes were observed, as well as polynuclear giant cells and at the site of wholly obliterated muscle fibers, islands of hyalinized connective tissue. In case 5 there was an increase in the number of sarcolemma cells together with complete disappearance of isolated muscle fibers; on the other hand, neither of these two cases presented any actual degeneration or necrosis. Largely the same structures



Fig 6 Case 7 Autopsy Liver Pronounced fatty degeneration and relatively advanced periportal cirrhosis.



Fig 9 Case 7 Autopsy Iliopsoas muscle Necrotic muscle fibers. Pronounced interstitial edema. Sparse accumulation of inflammatory cells.

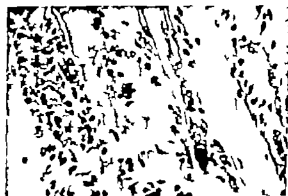


Fig 7 Case 6. Biopsy Left gluteal musculature To the left necrotic muscl fiber with granular disintegration of the sarcoplasm To the right hyalinized and partially fragmentary muscle fiber

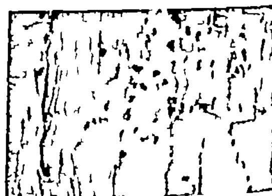


Fig 10 Case 8 Biopsy Musculature left thigh. Segment of a muscle fiber shows Zenker's degeneration.



Fig 8. Case 7 Biopsy Left biceps brachii. Groups of muscle fibers showing complet necrosis with obliteration of the striation, swelling and disintegration. Karyorrhexis and karyolysis.



Fig 11 Case 7 Autopsy Kidney Dilated tubuli with low epithelium. I several places compact cylinders of undefinable nature

were present in the biopsy specimens from cases 9 and 10.

In case 11 no definite histologic changes were observed.

Histologic examination of the kidneys

In summary the renal changes in the autopsy cases were those of lower nephron nephrosis. In all three cases deposits of a substance of undetermined nature were encountered in the renal tubules (fig 11)

Discussion

Symptoms and local muscle observations

The symptom picture was dominated by aching, tenderness and, in the majority of cases, edema both muscularly and subcutaneously The edema, which was often rather extensive, gave the symptom picture in some of the cases a certain resemblance to deep phlebothrombosis. *It* was demonstrated both by phlebography and at autopsy however that thrombosis was not the cause of the symptoms but rather that they were the *result of localized muscle necroses.*

Neurologic examination

In three cases (6 10 and 11) neuritis was present in addition to the muscle damage. It is uncertain, however if the nerve and muscle lesions have the same pathogenesis, considering that the somatic manifestations of chronic alcoholism are extremely complicated. For example, it is very difficult to evaluate the significance of possible nutritional deficiencies at the occurrence of the different symptoms.

Urine and renal function

In the presence of necrotic changes in the skeletal musculature, *renal damage* with anuria-oliguria and elevation of the non-protein nitrogen level may arise. The striated musculature contains appreciable quantities of potassium in comparison with the blood, wherefore there is also a risk of hyperpotassemia in cases of acute muscle necrosis. This risk is especially imminent in cases of concurrent renal impairment with anuria-oliguria. For instance, the trauma resulting from electric shock accidents (17) and carbon monoxide poisoning (6 7) sometimes gives rise to this mechanism with muscle necrosis, renal damage and sudden death from hyperpotassemia. Five patients in the material presented here had renal impairment with anuria oliguria and elevated non-protein nitrogen. In three of the five cases the serum potassium level was checked and found in all three to be appreciably elevated, with a maximal value of 12.4 mEq immediately before death.

In four cases myoglobin was demonstrated spectroscopically in the urine. There is no relation between the degree of myoglobinuria and the presence of renal impairment, however either in the cases in the present material nor in other forms of myoglobinuria. In the familial form of paroxysmal myoglobinuria (18) for example grave renal damage has never been demonstrated, although vast quantities of myoglobin can occur in the urine during the attacks. Thus, the renal impairment is in all probability n t directly caused by the myoglobin liberated from the disintegrating muscle cells.

By analogy with the mechanism of renal damage occurring in the crush syndrome (19 20) for example, it seems likely to us that acute necrosis of the skeletal musculature is a prerequisite for the origin of renal damage in the cases described here as well These muscle necroses probably do not need to be



Fig 6 Case 7 Autopsy Liver Pronounced fatty degeneration and relatively advanced periportal cirrhosis.



Fig 9 Case 7 Autopsy Iliopsoas muscle. Necrotic muscle fibers. Pronounced interstitial edema. Sparse accumulation of inflammatory cells.

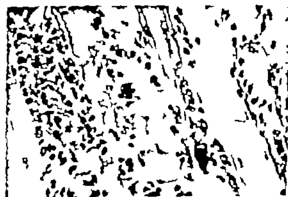


Fig 7 Case 6. Biopsy Left gluteal musculature To the left necrotic muscle fiber with granular disintegration of the sarcoplasm To the right hyalinized and partially fragmentary muscle fiber



Fig 10 Case 8. Biopsy Musculature left thigh. Segment of a muscle fiber shows Zenker's degeneration.



Fig 8. Case 7 Biopsy Left biceps brachii Groups of muscle fibers showing complete necrosis with obliteration of the striation, swelling and disintegration. Karyorrhexis and karyolysis.



Fig 11 Case 7 Autopsy Kidney Dilated tubuli with low epithelium In several places compact cylinders of undefinable nature

opsy specimens with the exception of case 7 were limited to certain, not very extensive sections and that in two cases (8 and 12) they appeared only as segmental necroses in isolated muscle fibers. This example illustrates the difficulty of obtaining representative muscle specimens for biopsy. It seems possible that this circumstance might have been the reason for the completely negative result of the muscle biopsy in case 11 notwithstanding that the patient presented unequivocal clinical symptoms from the musculature in the region of the excision at the time biopsy specimens were taken and also had myoglobinuria and an enzyme pattern resembling that in muscle damage with high LD and aldolase activity (fig. 5).

In our opinion the empty sarcolemma tubes encountered in case 3 might conceivably be interpreted as residues after necrosis of isolated muscle fibers. In cases 5, 9 and 10 where the principal observations were an increase in the number of sarcolemma nuclei and signs of obliteration of isolated muscle fibers the biopsy was not undertaken until after the clinical symptoms had begun to subside. There is of course no reason to expect representative histologic picture in these cases, and thus possibly the time factor can influence the biopsy results. The observations in case 5 are particularly interesting as that patient had manifest myoglobinuria, a phenomenon indicating disintegration of sarcoplasm. It therefore seems to us that the rather slight histologic changes in these three cases can be explained as residual signs of earlier muscle disintegration.

In the cases presented here we observed polynuclear giant cells, nuclear conglomerates or so-called myocytes rather often. In our opinion, however we have not observed definite regeneration phenomena.

Histologic examination of the kidneys

The renal changes in the autopsy cases had the character of lower nephron nephrosis with cylinders present in the tubuli. The question naturally arises whether these might have contained myoglobin, but as no analysis of this substance was possible, the nature of the cylinders is uncertain.

Pathogenesis

It is our opinion that there is a relation between the muscle damage and the alcohol abuse in the cases reported here as the muscle symptoms described in this series occurred exclusively in advanced cases of chronic alcoholism. On the other hand, it is naturally difficult to picture the pathogenic mechanism of the muscle damage. It is evident from veterinary medicine that a number of different deficiency disorders are known to be able to give rise to muscle damage.

In dietic hepatosis in pigs, which is caused by a deficiency of certain amino acids in the diet, Obel found waxy degeneration in the skeletal musculature in 60 per cent (25). Similar changes in the skeletal musculature can also be induced experimentally in rabbits if they are placed on a choline-poor diet (26). Different types of avitaminosis can give rise to muscle damage in domestic animals. Vitamin E deficiency in lambs can induce a pathologic condition called white muscle disease or stiff lamb disease (27). A similar condition occurs in calves as a result of vitamin C deficiency (28).

It is quite conceivable that different deficiency disorders giving rise to muscle damage occur in human medicine as well, although pure deficiency conditions

especially extensive to give rise to impaired renal function In case 6 for instance, so far as could be determined from the clinical findings and the histologic examinations, the necrosis was localized to the left thigh and gluteal musculature only (biopsy specimens from the right thigh showed a normal picture) but nevertheless there was grave renal damage. Moreover no myoglobinuria was demonstrable in this case.

Liver muscle enzymes in serum

An appreciable increase in the transaminase activity in the serum often occurs in chronic alcoholics after a period of excessive alcohol consumption (21 22, 23 24) In the cases discussed here the activity in the serum of a number of different liver muscle enzymes was also considerably elevated. This applies particularly when the determinations were carried out during the acute stage. The GPT rise, which was appreciable in a number of cases, definitely indicates that liver-cell necrosis must have occurred. On the other hand the appreciable LD and aldolase increase that occurred in some of the foregoing cases is evidence against the liver as the sole source of the increased enzyme activity An increase in LD and aldolase activity of the order of magnitude that occurred in this material never arises in cases of liver necrosis only

For the aforementioned reasons the increases in enzyme activity in the cases discussed here were probably caused by necrosis in both the liver and the muscles

Liver biopsy

At liver biopsy histologic changes with fatty degeneration or cirrhosis of the liver were demonstrated in all except three cases (2, 3 and 6) In these three cases, however biopsy was not carried out until one to two months after the acute onset of symptoms. In case 6 at the time of admission to the hospital the GPT activity in serum had risen to over 200 units. Therefore in that case damage to the liver was in all probability present at the time, although biopsy one month later showed a normal picture. In the other two cases presenting normal liver biopsy results the GPT activity in serum was not determined during the acute stage.

Histologic examination of muscle specimens

The histologic observations in the examinations of skeletal musculature varied widely In seven cases (1 2 4 6 7 8 and 12) Zenker's degeneration was commonly found. In several cases disintegration or granulation of the sarcoplasm was also noted. In the autopsy cases an exceedingly pronounced general disintegration of large muscle segments was found. In one of the autopsy cases (7) an earlier muscle biopsy specimen showed pronounced and essentially the same changes as were subsequently observed in the autopsy material. Another change characteristic especially of the autopsy cases, was very extensive muscular edema. In several patients accumulations of inflammatory cells were encountered in the musculature. These were not very marked however and we interpreted them as a reaction to disintegration or degeneration of muscle fibers rather than as signs of myitis in the true sense.

That the histologic changes in the musculature should be most extensive in the autopsy cases was, of course to be expected At the same time it should be pointed out that the changes in the bi-

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are less common. It should be mentioned, however, that Popper observed grave myocardial lesions in young chronic alcoholics and interpreted the condition as due to protein deficiency (29). Similar myocardial conditions have been demonstrated in Bantu Negroes without there having been alcohol abuse; protein deficiency was believed to have been the cause (30). The skeletal musculature has not been histologically examined in these conditions in man. In beriberi Wenckebach (31) observed histologic changes in the skeletal musculature in the form of thick, swollen muscle fibers, which he interpreted as a parallel phenomenon to the myocardial lesion resulting from vitamin B_1 deficiency.

In the patient with lesions resulting from chronic alcoholism the prerequisites for the occurrence of different deficiency symptoms are present. The polyneuritis in alcoholics for example is generally regarded as due to vitamin B_1 deficiency (32, 33) and the liver damage to protein deficiency in combination with the toxic effect of the alcohol (34).

A pathogenic relation between liver necrosis and waxy degeneration in the skeletal musculature is reported in the literature (35). For instance, in experimental studies when animals are placed on a diet poor in choline or vitamin E, both liver and muscle damage occur (36). Such is the case in dietic hepatosis in pigs (25).

In the present material muscle damage and liver damage occurred concurrently as a rule. The relation between these two is naturally difficult to define, but the observations cited here, particularly those from veterinary medicine, seem to us to justify interpretation of the muscle lesions as a parallel phenomenon to the usual liver changes in chronic alcoholism.

Summary

Twelve cases presenting a symptom complex in chronic alcoholism not previously described are reported. All patients (2 women, 10 men) were advanced alcoholics, and the onset of the symptoms followed a period of high-grade alcohol abuse.

The symptoms were aching and tenderness in the musculature and in most cases muscular and subcutaneous edema, leading in several cases to suspicion of incipient deep phlebothrombosis.

In five cases renal damage was present with anuria and elevated non-protein nitrogen. Three of these patients had hyperpotassemia.

Four patients had spectroscopically demonstrated myoglobinuria.

The GOT, GPT, LD and aldolase activity in serum showed a pattern indicating necrosis in both liver and musculature.

Muscle biopsy showed degeneration and necrosis of muscle fibers in most of the cases.

The pathogenesis of the muscle changes is discussed and the possibility of a common pathogenesis for the liver and muscle changes is suggested.

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Intrinsic Factor Deficiency, Achlorhydria, and Malignancy in Polyps of the Stomach and Duodenum

By

O Eklöf L. Ekstedt and P Reizenstein

The number of patients with idiopathic pernicious anaemia (PA) in whom carcinoma of the stomach develops is estimated by different authors as between 3 and 21 times that which might be expected from random distribution (30, 42, 50, 57 65 67 76) Gastric polyps have furthermore been found in a strikingly large proportion of cases in many series (10, 30 37 57 61 67) But despite the apparently indubitable connexion between PA and the development of gastric neoplasms of epithelial origin, relatively few patients with gastric tumour develop manifest megaloblastic anaemia. In carcinoma of the stomach, where the frequency for understandable reasons is lowest, this incidence is reported to range from 0.6 to 3.1 per cent (37 58, 73) and in polyps from 3 to 23 per cent (13 17 31 37) These variations may be presumed to be due to the differing compositions of the series and diagnostic difficulties.

The collected polyp material at Karolinska sjukhuset permitted a study on the relationship between intrinsic factor (IF) deficiency achlorhydria, and malignancy in polyps of the stomach and duodenum. The purpose of the study was to ascertain

To what extent is IF-deficiency present in polyps of the stomach or duodenum?

What is the incidence of achlorhydria?

Is the number situation, or size of the polyps of any significance for the development of IF-deficiency and/or achlorhydria.

Are patients with gastric or duodenal polyps particularly susceptible to cancer?

What is the significance of IF-deficiency and achlorhydria in the development of cancer?

Material and methods

The series comprised 176 patients with abdominal disorders in whom one or more polyps had been diagnosed in the stomach or duodenum. It was collected through scrutiny of roentgen films and examination of case records, operative, gastroscopic, and autopsy reports for the years 1940 to 1960 inclusive. All

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Table II Sex distribution and mean age in the series as a whole and in different groups when diagnostic tools were employed

Group	No. of patients	Sex distribution M/F	Average age (years)
Entire series at roentgen diagnosis of polyp	176	82/94	62.3
Patients with malignant tumour or premalignant changes when malignancy was diagnosed	64	35/29	66.9
Cases of PA diagnosed with methods other than URT	8	0/8	64.0
Patients examined with URT	45	20/25	63.1
Patients with normal URT-values	31	14/17	60.9
Patients with IF-deficiency demonstrated at URT	14	6/8	68.0
Patients examined with test meal	101	42/59	60.8
Patients with free hydrochloric acid on test meal	16	6/10	54.8
Patients with achlorhydria on test meal	85	36/49	62.0
Patients with achlorhydria and IF-deficiency demonstrated at URT and/or idiopathic PA	17	3/14	65.0

production of IF and free hydrochloric acid was normal (see also tables IX and X)

IF-deficiency in polyps of the stomach or duodenum

Of the 45 patients examined with URT 14 had a 48-hour-excretion of $Co^{60}B_{12}$ of 11 per cent or less of the test dose administered (table III). In case KH impaired renal function, and in cases KA and LK earlier partial gastric resection cannot be ruled out as a subsidiary or the chief cause of the low URT-value. The 11 remaining patients with pathologic URT-values included four in whom the value was of an order associated by experience with idiopathic PA, but only one patient (HA) had manifest megaloblastic anaemia at the time of the examination. The patient KA, mentioned above, has received treatment for this anaemia and now has an almost normal peripheral blood picture.

Table III Results of URT in 14 cases in which the 48-hour excretion of $Co^{60}B_{12}$ was suggestive of IF-deficiency

Case	Percentile excretion of $Co^{60}B_{12}$ during 48 hours	
	URT without IF in %	URT with IF in %
WA	0.7 + 0.2	18 + 6
HA	1.5 + 3	21 + 7
SM	3 + 1	19 + 5
SA	4 + 3	12 + 5
KAM	9 + 0	12 + 5
TE	7 + 4	13 + 5
SG	7 + 4	13 + 5
JW	7 + 4	15 + 5
EE	7 + 4	18 + 6
KH	6 + 3	22 + 6
KA	0.6 + 0	4 + 0
LK	4 + 3	4 + 1
AD	2 + 0.2	1 + 1
FF	7 + 4	10 + 5

The patient had impaired renal function.
The patient had undergone Billroth II resection.
Slight or no reaction to administered IF

Table I Causes of death in 87 cases of polyps of the stomach or duodenum

Carcinoma of the stomach (malignant polyps)	24
Carcinoma of other situation	27
Circulatory disease	25
Respiratory disease	5
Urinary tract disease	2
Liver disease	3
Traffic accident	1
	87

[1] See also table VI

the 176 patients had been examined roentgenographically once or more often. Nine cases in which the roentgen findings were verified histologically at operation, and one in which gastroscopy furnished gross verification, are included although no roentgen films were available for re-examination. The diagnosis of polyp was in another 58 cases verified histologically at biopsy in seven cases gastroscopically and in ten at explorative laparotomy or at autopsy In the remaining 89 cases, the diagnosis was based solely on the roentgen findings however we did our best to include only cases in which the roentgen appearances left no room for doubt and in which there was otherwise nothing to refute the diagnosis.

Eighty-seven of the patients have succumbed. The cause of death (table I) was in 26 instances established at autopsy and in 61 on the basis of clinical observations. In 23 of the latter cases, the clinical diagnosis was confirmed by biopsy and in four by the findings of explorative laparotomy

Of the 176 cases in the series, 122 have earlier been presented as regards diagnosis and therapy by Eklöf et al. (1960)

The incidence of IF-deficiency in the series was studied indirectly with the aid of the so-called Schilling test (urinary retention test, URT) Forty-five patients volunteered for this examination.

The URT was performed according to the modification described by Reizenstein (1959) The patients arrived in the fasting state. Fifteen minutes after a subcutaneous injection of 0.25 mg of carbacholin, the radioactive B_{12} (approximately 0.50 micrograms of $Co^{60}B_{12}$) was taken by the mouth, and after another two hours an intramuscular injection of 1 000 micrograms of vitamin B_{12} was given. All the urine was collected for 24 hours, and a further intramuscular dose of B_{12} of the same size was administered. The urine was again collected for 24 hours, after which the total amount of radioactive B_{12} excreted was determined.

A 48-hour-excretion of $Co^{60}B_{12}$ of 12 per cent or more of the test dose has been regarded as normal (59) In instances in which the URT values were at this level or below it, the examination was repeated and IF administered simultaneously Patients with such values were also examined neurologically and their blood picture fully investigated — including determination of the serum B_{12} level (measured with Euglena gracilis z)

The main series included 8 patients with idiopathic PA earlier diagnosed with conventional methods (peripheral blood picture bone-marrow changes, initial response to therapy achlorhydria, et cetera) who were comparable to those with pathologic URT values (a ninth patient, KA in table III, was also examined with URT)

The incidence of achlorhydria was estimated on the basis of 101 patients who at some time during the course of their disease — usually in connexion with the diagnosis of polyp — had been examined with a test meal. Of these, 42 were given Ewald's and 59 a histamine test meal.

The number situation, and size of the polyps were evaluated on available roentgenograms.

The incidence of malignant tumours in the series was studied in case records, operative and autopsy reports, and in some instances with the help of information from private practitioners.

Results

The series comprised 82 men and 94 women with a mean age of 62.3 years when polyp was diagnosed. This was roughly the same in men and women. The sex distribution and mean age in different groups discussed below are given in table II As is seen there, patients with IF-deficiency and achlorhydria were in general older than those in whom the

Table V. Distribution of cases according to size of polyps, as measured on the roentgenograms, and malignant degeneration (malignant gastric tumours) in the series as a whole and in different groups

Group	No. of cases	Polyps < 2 cm	Malignancy	Polyps ≥ 2 cm	Malignancy
Whole series	166	115	6	51	*25
Patients with normal URT	31	25	0	6	0
Patients with URT-values suggestive of IF-deficiency and/or idiopathic PA	21	16	0	5	3
Patients with free HCl	16	11	0	5	2
Patients with achlorhydria	76	47	*6	29	13
Patients with achlorhydria, IF deficiency at URT and/or idiopathic PA	15	11	0	4	2

Ten cases in which roentgenograms were not available were rejected, including three patients with polyps affected with malignant degeneration.
One case in which no roentgenograms were available was rejected.
Nine cases in which roentgenograms were not available were rejected, including two patients with polyps affected with malignant degeneration.
One patient with premalignant changes included.

whole and in various groups are given in table IV. A solitary polyp in the fundus of the stomach was specially recorded on roentgen examination in only one instance and the surgeons did not find any further polyps of this situation in the operation cases.

Fifty-one of the patients had polyps with a diameter exceeding 2 cm as measured on the roentgenograms (table V).

Malignancy in polyps of the stomach or duodenum

Altogether 66 malignant gastric or extragastric tumours were demonstrated in 62 of the patients in the series. In two of them there were also premalignant changes sited elsewhere. In another two patients only premalignant changes were present. The diagnosis (table VI) was in 50 cases verified histologically at operation in nine cases it was based on the gross findings at explorative laparotomy or autopsy in 11 instances it was founded on the roentgen appearances and the course of the disease.

Table VI. Situation of 66 malignant tumours and four premalignant lesions in 176 patients with polyps of the stomach or duodenum

Carcinoma of the nasopharynx	1
Carcinoma of the oesophagus	3
Carcinoma of the stomach (malignant polyps)	34
Carcinoma of the small intestine	1
Carcinoma of the colon	*6
Carcinoma of the biliary passages and liver	3
Carcinoma of the pancreas	2
Carcinoma of the peritoneum (malignant cells in ascites)	1
Carcinoma of the lung	3
Carcinoma of the breast	4
Carcinoma of the cervix uteri	2
Carcinoma of the ovary	*2
Carcinoma of the prostate	2
Carcinoma of the renal pelvis	1
Carcinoma of the skin	
Leukaemia	3

Two cases of premalignant lesions included.
One case of premalignant lesions included.

Table IV Number and situation of polyps in the series as a whole and in various groups

Group	Polyposis	Multiple polyps of gastric body	Multiple polyps of pyloric canal	Solitary polyp of gastric body	Solitary polyp of pyloric canal	Duodenal polyp
Whole series	39	42	18	26	38	13
Patients with normal URT	8	6	2	4	5	6
Patients with URT values suggestive of IF-deficiency and/or idiopathic PA	2	3	1	4	10	2
Patients with free HCl	2	4	1	1	7	1
Patients with achlorhydria	26	25	8	10	14	2
Patients with achlorhydria, IF deficiency at URT and/or idiopathic PA	2	2	1	2	8	2

Multiple duodenal polyp in one case.
Polyp situated in the fundus of the stomach in one case.

Four other patients who had undergone partial gastric resection for polyps, and ten in whom polyps had been excised, had normal URT values — in some instances near the 12 per cent limit.

Neurologic and haematologic examination of patients with low URT values revealed nothing deviating from normal — with the exception of case HA. Since this part of the follow-up investigation was carried out after URT at intervals ranging from a few weeks to twelve months, it cannot be determined how many patients at the time of the URT examination had blood changes of megaloblastic type — although these were no longer demonstrable. It may be noted that ten of the patients with the URT values under discussion had at periods been treated for anaemia but it was only in cases HA and KA that the anaemia was known to have been of megaloblastic type. Pathologic values in serum B_{12} determinations were recorded in patients KA and LK, mentioned above (64 $\mu\mu$g/ml and 90 $\mu\mu$g/ml respectively) who had undergone Billroth II resection 15 and 12 years earlier The significance of the resection is also in this respect somewhat obscure. In these two cases, the serum B_1 was determined approximately twelve months after URT and both patients had in the meanwhile received certain anti-anaemic therapy including liver preparations.

Achlorhydria in polyps of the stomach or duodenum

Achlorhydria was recorded in no less than 85 of the 101 patients given a test meal, while free hydrochloric acid was found in 16 cases (table II)

Number situation, and size of the polyps

Solitary polyps were present in 76 patients and multiple polyps (two or more) in 100 In 39 of the cases of multiple polyps the lesions were so extensive as to be termed polyposis. The number and situation of the polyps in the series as a

Table V. Distribution of cases according to size of polyps, as measured on the roentgenograms, and malignant degeneration (malignant gastric tumours) in the series as a whole and in different groups

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One patient with premalignant changes included

whole and in various groups are given in table IV. A solitary polyp in the fundus of the stomach was specially recorded on roentgen examination in only one instance, and the surgeons did not find any further polyps of this situation in the operation cases.

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Altogether 66 malignant gastric or extragastric tumours were demonstrated in 62 of the patients in the series. In two of them there were also premalignant changes sited elsewhere. In another two patients only premalignant changes were present. The diagnosis (table VI) was in 30 cases verified histologically at operation. In nine cases it was based on the gross findings at explorative laparotomy or autopsy in 11 instances it was founded on the roentgen appearances and the course of the disease.

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Carcinoma of the peritoneum (malignant cells in ascites)	1
Carcinoma of the lung	3
Carcinoma of the breast	4
Carcinoma of the cervix uteri	2
Carcinoma of the ovary	*2
Carcinoma of the prostate	2
Carcinoma of the renal pelvis	1
Carcinoma of the skin	2
Leukaemia	3

Two cases of premalignant lesions included.
One case of premalignant lesions included.

Table VII Distribution of cases of malignant gastric tumour in relation to the number and situation of the polyps

No and situation of polyps	No of cases	Distribution of malignant tumours of the stomach
Polyposis	39	15 (38%)
Multiple polyps of the body of the stomach	42	8 (19%)
Multiple polyps of the pyloric canal	18	4 (22%)
Solitary polyp of the body of the stomach[1,2]	26	6 (23%)
Solitary polyp of the pyloric canal	38	1 (3%)
Duodenal polyp	13	0 —

One solitary polyp was situated in the fundus of the stomach.
One case of premalignant changes included.
Multiple duodenal polyps in one case.

Table VIII Distribution of cases of malignant gastric and extragastric tumours in relation to the number and situation of the polyps

No. and situation of polyps	No. of cases	Distribution of malignant gastric and extragastric tumours
Polyposis	39	21 (54%)
Multiple polyps of the body of the stomach	42	21 (51)
Multiple polyps of the pyloric canal[2,3]	18	11 (61%)
Solitary polyp of the body of the stomach	26	9 (35%)
Solitary polyp of the pyloric canal	38	6 (16%)
Duodenal polyp	13	2 (15%)

One case of premalignant changes included.
One solitary polyp was situated in the fundus of the stomach.
Two cases of premalignant changes included.
Multiple duodenal polyps in one case.

Thirty two patients had malignant, and two premalignant gastric lesions which in 25 instances had a diameter of 2 cm or more (table V) In six cases the malignant gastric tumour was smaller and in three there were no preoperative roentgen films for evalutaion

Malignant and premalignant changes in the stomach occurred in equal incidence in the two sexes (18 men and 16 women) so that the slight male predominance of cancer (table II) in the series as a whole is attributable to a larger number of extragastric tumours in the men

Malignant degeneration was not recorded in any case of duodenal polyp Malignancy was found only in one case of solitary polyp of the pyloric canal (table VII) while there was a much higher and roughly equally high incidence of malignant gastric tumours in all other forms of polyp — except in polyposis in which the incidence was almost twice as high. It would also seem that as regards general susceptibility to malignant tumours, patients with duodenal polyps or solitary polyps of the pyloric canal are in a safer position than those with multiple polyps (table VIII) Patients with solitary polyps of the body of the stomach come between the two groups in this connexion.

The incidence of carcinoma of the stomach among patients with IF-deficiency and/or PA was somewhat lower than the average incidence in the series as a whole while in achlorhydria this incidence was somewhat higher than the average (table V)

Discussion

Polyps of the stomach and duodenum are reported by most authors to be about equally common in the two sexes (20

31 34 37) The sex distribution in the present series did not, then, deviate from the usual. But women predominated among the patients with IF-deficiency and/or manifest megaloblastic anaemia (6 men to 16 women) a sex distribution often recorded in PA (12, 36, 37 51 68).

The mean age of the patients when the diagnosis of polyp is made ranges in most series between 50 and 60 years, but even exceeds 65 years in some (17 20 25, 31) In the present series, then, the mean age lies at the upper limit of the reported range. Advanced age seems to be of some significance for the development of both IF-deficiency and achlorhydria.

The composition of the series, elderly patients with a fairly high incidence of disease and a high mortality must lead one to assume that the patients agreeing to undergo URT and associated examinations belong to the subjectively and probably also objectively healthier of the surviving patients. This in some measure limits the conclusions which may be based on the URT results, since it seems likely that cases of malignant tumour in particular are under-represented in this part of the series.

The relation between age and URT values is illustrated in table IX, which shows that the patients with normal values were significantly younger (0 01 > p > 0 001) than those with "low normal values" or pathologic values. There was no difference between the latter two groups.

Opinions differ as to the existence of any correlation between URT values and age, the values decreasing with advancing age but there is much t suggest this to be so. A falling percentile excretion of the test dose in older patients was demonstrated at URT in a series of healthy persons by Watkin et al. (1953) but this result could not be reproduced by Schilling (1955) Chow et al. (1956) or Tauber et al. (1957) The existence of an age correlation is suggested by the demonstration by Ylvisaker et al. (1955) and others at gastric biopsy that the number of cases of atrophic gastritis increases with advancing age, and Glass (1956 and 1957) Gräsbeck & Siurala (1958) Siurala, Erämaa & Nyberg (1960) and others found a distinct tendency to lower URT-values in patients with atrophic gastritis. This tendency on the whole paralleled the severity of the atrophic gastritis.

Table IX. Relation between $Co^{60}B_{12}$-absorption and age in 45 patients with polyp of the stomach or duodenum

Results at URT (% of $Co^{60}B_{12}$ administered in 48-hours urinary excretion)	No. of cases	Mean age in years ± S. D.	Degree of significance
≥ 20	18	56 ± 3.2	
12–19	13	68 ± 1.67	0.01 > p > 0.001
0–11	14	68 ± 1.48	

As mentioned earlier the patients with pathologic URT-values included one with impaired renal function (KH in table III) and two (KA and LK) who had undergone Billroth II resection. Brodine et al. (1959) and Lous & Schwartz (1959) found IF-deficiency to be more common following partial gastrectomy than was formerly supposed. This probably derives largely from the mucosal changes which n most instances accompanied the basic disease (5 41)

If the three patients mentioned above in whom extraneous factors may have

affected the URT values are excluded from the analysis of the incidence of IF deficiency in patients examined with URT this deficiency was demonstrable in approximately one fourth of cases. This is a strikingly high frequency The low URT values were probably due to the atrophic changes in the gastric mucosa commonly associated with polyps (7 30 32, 40 57 75) Whether or not patients with a normal peripheral blood picture but with pathologic URT values — if not treated — in time develop manifest megaloblastic anaemia remains open to question (2 4 11 41 47 64) However it is our intention to give these patients continuous B_{12} therapy

The five patients in whom the excreted amount of $Co^{60}B_{12}$ at URT with IF showed only a slight rise, or none at all, included patients KH, KA, and LK (table III) The impaired renal function in case KH may once again provide the explanation. Patients KA and LK, like AD and FF had periodically received peroral B_{12} or liver therapy Experience has also shown that such therapy causes resistance to porcine IF There was no evidence of malabsorption.

The incidence of achlorhydria recorded in the series corresponds fully with earlier observations, in which 80 per cent or more of the patients with polyps lack free hydrochloric acid (17 20 31) A higher frequency of achlorhydria is seen only in idiopathic PA, when free hydrochloric acid if anything refutes the diagnosis. In carcinoma of the stomach, the figures in different series range from about 50 per cent to 85 per cent (1 19 26 52 56) In patients of the same ages without any known tumour of the stomach, achlorhydria has been demonstrated in between 19 and 35 per cent of cases (26 33 77 80) The figures in table X suggest a correlation between age and gastric acidity in the series. There was a significant age difference ($0.05 > p > 0.02$) between the patients with free hydrochloric acid and those with achlorhydria. The latter then were generally somewhat older An even greater difference in age ($0.02 > p > 0.01$) existed between the patients with free hydrochloric acid and those with achlorhydria, IF-deficiency and/or idiopathic PA. In patients with polyps of the stomach or duodenum, free hydrochloric acid would seem to be present mainly in patients below the age of 60 years.

The fact that the test meal in some two-fifths of cases was Ewald's may warrant some criticism. This was the test meal used at the hospital during the greater part of the forties. Since most of the patients examined at that time have died, no new test with a histamine meal is possible. It may also be noted that in some patients with free hydrochloric acid, the test meal was given several years before the detection of polyps and does not necessarily reflect later conditions.

No distinct relationship was noted between, on the one hand, the number situation, and size of the polyps and, on the other hand IF-deficiency and/or manifest PA or achlorhydria (table IV)

The relation between the size of the polyps and the danger of malignancy is of special interest. Rieniits & Broders (1946) and Hay (1951) found malignancy in cases having polyps to be appreciably more common if the diameter of the polyps exceeded 2 cm. This observation has since been confirmed by several authors (6 14 20 29) and agrees well with our own experience (table V)

The link between IF-deficiency idiopathic PA, achlorhydria, polyps of the

Table X. Relation between acidity of the stomach and mean age in 101 patients with polyps of the stomach or duodenum

Acidity of the stomach	No of cases	Mean age in years ± S. D.	Degree of significance
Patients with free HCl	16	55 ± 2.93	$0.05 > p > 0.02$
Patients with achlorhydria	85	62 ± 1.14	
			$0.02 > p > 0.01$
Patients with achlorhydria, IF-deficiency at URT and/or idiopathic PA	17	65 ± 2.29	

Age correlation also between 16 patients with free HCl and 17 patients with achlorhydria plus IF deficiency and or idiopathic PA.

stomach or duodenum and carcinoma of the stomach may be atrophic gastritis, but the causal connexion is as yet not fully understood. Atrophic mucosal changes have been demonstrated at biopsy in almost every case of PA (10 18, 43 44 45, 68) and are widely reported to be associated with carcinoma of the stomach (30 41 37 69 72) and with polyps (7 30 32, 40 57 75)

Many authors have found the degree and distribution of the trophic mucosal changes to be directly correlated with the ability of the stomach to produce hydrochloric acid and IF Accordingly pathologic results of test meals and URT should suggest gastric mucosal atrophy The ability to secrete hydrochloric acid usually disappears first, while the production of IF does not cease until the mucosal changes have progressed — at a later stage (34 33 68) This is in some measure confirmed by the results in our study (tables II, IX and X)

Just as atrophy (and intestinal metaplasia) of the gastric mucous membrane is said to occur in cases of duodenal ulcer (48, 49 7?) although to a lesser extent than in gastric ulcer and carcinoma, it seems likely to us that atrophic mucosal lesions in the stomach can be expected in the presence of duodenal polyps. Achlorhydria and IF-deficiency were demonstrated in our series to almost the same extent in cases of duodenal polyps as, for instance, in the presence of solitary polyps in the body of the stomach or the pyloric canal.

Although most authors (7 32, 38, 41 48 49 70, 71) regard gastric polyps as definitely precancerous changes, this concept has been questioned. Plachta & Speer (1957) Brealaw (1960) and others maintain that malignant polyps are primarily malignant, and that no transition from benign to malignant polyp has been demonstrated conclusively Pollard (1959) Joly & McNeer (1959) and Huppler et al. (1960) did, however find much circumstantial evidence that carcinoma may arise in a benign polyp and Mider (1960) regarded the whole discussion as largely academic since it has practically no significance for the clinical management of these patients. Malignant degeneration of duodenal polyps has not

affected the URT-values are excluded from the analysis of the incidence of IF deficiency in patients examined with URT this deficiency was demonstrable in approximately one fourth of cases. This is a strikingly high frequency The low URT values were probably due to the atrophic changes in the gastric mucosa commonly associated with polyps (7 30 32 40 57 75) Whether or not patients with a normal peripheral blood picture but with pathologic URT values — if not treated — in time develop manifest megaloblastic anaemia remains open to question (2 4 11 41 47 64) However it is our intention to give these patients continuous B_{12} therapy

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Our figures suggest that polyps may be the manifestation of a generally increased *susceptibility to tumour* with an indubitable tendency to malignant degeneration (cf. Rienits & Broders 1946 and Berg 1958) If on the basis of the Swedish cancer statistics (62) the incidence of carcinoma in the present series is compared with that in a comparable population group statistical testing (79) *reveals a highly significant difference* between the two

The probability is slight that the high incidence of carcinoma in the series was attributable to random distribution ($p \leqslant 0.001$) The very marked tendency to develop malignant tumours noted in cases of polyp of the stomach or duodenum is not limited only to malignant degeneration of the polyp or to the coexistence of polyp and primarily malignant gastric tumours, *as is exemplified by the fact* that the number of extragastric tumours was also highly significantly elevated — although to a lesser degree ($p < 0.001$)

The number of malignant tumours in the patients with IF-deficiency and/or idiopathic PA was lower than the average for the series as a whole this may be due in part to random distribution in a small group and in part to the patients in this group deviating in some measure *from the rest of the series, as mentioned* earlier In any event, there was no evidence that these patients were more liable to cancer *than others with polyps.*

Nor did the series afford any support for the assumption that gastric acidity is of decisive importance for the development of malignant tumours in polyp patients However the group of patients with free hydrochloric acid is too small to permit any final statistical evaluation

Summary

A series of 176 patients with polyps of the stomach or duodenum were analyzed as to the relationship between intrinsic factor (IF) deficiency achlorhydria, malignancy and these polyps.

The mean age of the patients at the time when the polyps were diagnosed was 62.3 years. Age is of some significance for the development of achlorhydria and possibly also for that of IF-deficiency each of which tends to *increase with advancing age.*

In approximately one-fourth of the 43 patients examined with the Schilling test (URT) values were obtained which were suggestive of IF-deficiency Of these patients only one had manifest megaloblastic anaemia at the time of the examination, while another who had been treated for anaemia of this type had an almost normal peripheral blood picture. *Megaloblastic anaemia was diagnosed* with conventional routine methods in another eight cases IF-deficiency with or without manifest megaloblastic anaemia was, then, a fairly common finding in the series. Consequently patients in whom polyps are diagnosed should always be examined with this in mind.

Achlorhydria was recorded in 85 of 101 patients examined with a test meal Free hydrochloric acid is rare in polyp patients, *being found chiefly* in those under the age of 60 years.

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Metabolism of Infused Albumin in Protein-losing Gastroenteropathy

By

Stig Jarnum

Albright, Bartter & Forbes (1949) investigated the fate of intravenously administered albumin in a case of idiopathic hypoproteinemia. In meticulous metabolic experiments they demonstrated that the mechanism behind the hypoproteinemia was one of hypercatabolism, i. e. an abnormally rapid breakdown of albumin. Their results were later confirmed in additional cases by means of I-labelled albumin. This led to the descriptive term "idiopathic hypercatabolic hypoproteinemia" introduced by Schwartz and Thomsen in 1957. The hypoproteinemia is due to excessive loss of plasma proteins in the gastrointestinal tract (Gordon 1959). Although the rapid elimination of infused albumin or plasma proteins in protein-losing gastroenteropathy[2] is well established, it is still unknown whether the artifically raised serum albumin concentration may influence the *degradation rate*. If so, it would throw light on the obscure process by which the abnormal gastrointestinal exudation is effected. The following investigations represent an attempt to clarify this through a comparison of the degradation rates of I-albumin and of infused albumin.

Material

Four patients were examined. Two men suffered from protein-losing gastropathy ("giant hypertrophic gastritis") and 2 (man and woman) from protein-losing enteropathy. One of these had a chronic exudative enteritis, the other an intestinal granulomatosis of unknown etiology. Clinical details have been published elsewhere (Schwartz & Jarnum 1961). In all of them an abnormal gastrointestinal permeability for macromolecules had been demonstrated by the aid of I-polyvinylpyrrolidone. In none of the patients was there any sign of renal or hepatic dysfunction.

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"Protein-losing gastroenteropathy" was suggested by The Lancet (1959) as "a descriptive, non-committal and brief" interim term.

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Table I Oral and parenteral nitrogen supply during metabolic investigations in 4 patients with abnormal gastrointestinal protein loss

Patient No	Sex	Age yrs	Diagnosis	Weight kg	Nitrogen supply		
					Oral g/kg/day	Intravenous (total)	
						g/kg	as albumin g kg
1	M	62	Giant hypertrophic gastritis	65	0.20	0.47	2.93
2	M	35	Giant hypertrophic gastritis	*62	0.12	0.55	3.43
3	F	28	Chronic exudative enteritis	47	0.20	0.66	4.14
4	M	38	Intestinal granulomatosis	65	0.22	0.55	3.45

Estimated non-edematous weight.

Table II Serum albumin concentration and the amount of circulating albumin in 4 patients with abnormal gastrointestinal protein loss before (I) 1—2 days after (II) and 8—11 days after (III) albumin infusions

Patient No.	Serum albumin g/100 ml			Circulating albumin g/kg			Total albumin g/kg	Increase of plasma volume during infusion %
	I	II	III	I	II	III	I	
1	2.61	4.29	3.48	1.16	2.34	1 79	2.76	23
2	0.99	2.77	1 63	0.42	1.58	0.85	1.00	34
3	2.31	4 63	3 43	1.03	2.18	1 49	2.45	3
4	2.01	3.52	2.26	0.97	1.79	1 12	2.38	5

Total albumin was estimated from circulating albumin assuming that 42 per cent of total albumin was intravascular (Jarnum & Schwartz 1960)

Methods

Serum albumin was determined by Kjeldahl analysis $\left(\frac{\text{protein}}{\text{nitrogen}} - \text{factor } 6.25\right)$ and paper electrophoresis (Laurell, Laurell & Skoog 1956)

Circulating (= intravascular) *albumin* was calculated from serum albumin concentration and the plasma volume determined by T 1824 or ^{131}I albumin. The plasma concentration of T 1824 was measured by direct photometry or by an extraction method (Jarnum 1959 a) In the former case a standard curve was constructed from the optical densities of T 1824 standard solutions in the patient's own serum. Plasma volume was calculated from the dilution 15 minutes after intravenous injection of T 1824 or ^{131}I-albumin. A factor of 0.98 was applied to correct for dye-loss during mixing time

^{131}I-albumin was prepared according to the method of Veall, Pearson & Hanley (1955)

I-albumin metabolism was taken as the average of the daily metabolic plasma clearances over a period of at least 8 days (Pearson, Veall & Vetter 1958) ^{131}I-contents of plasma, urine and homogenized feces were measured in a NaI thallium-activated scintillation detector

Metabolic investigations

The patients were subjected to a metabolic regime with a fixed diet for a period of 2—4 weeks (table I). In 3 patients the diet supplied 0.2 g nitrogen per kg per day, in the fourth (no. 2) only 0.12 g/kg/day because his appetite was small. All patients had 30—40 calories per kg per day.

Identical duplicates were made of all meals and collected through periods of three days, after which they were homogenized in blender. The contents of nitrogen, sodium and potassium were determined (Kjeldahl analysis, respectively flame photometry) in food, urine and feces (after homogenization).

After an initial experimental period of 3 to 15 days a total of 3—4 g albumin per kg was given intravenously in the course of 3 days. The albumin preparation applied[1] contained considerable amount of sodium (250—300 mEq per liter). This was probably the reason why no constant reduction of the patients' weight was observed during and after the infusions.

Serum albumin and the amount of circulating albumin (CA) was determined before, 1—2 days after and 8—11 days after the infusion period.

Results

Case 1. Male, 62 years. Giant hypertrophic gastritis.

This patient had no severe hypoalbuminemia at the time of this investigation (serum albumin 2.61 g/100 ml) and only slight crural edema. In the initial period he maintained nitrogen equilibrium (fig. 1). Infused nitrogen was retained during the infusion period, but 10 days later one third was excreted according to the cumulative nitrogen balance in fig. 1.

Serum albumin and CA increased 64 and 102 % respectively during the infusion period. Ten days later they were still 33 and 55 % above the preinfusion level (table II). The accidental weight loss was 4 kg irrespective of the cumulative sodium balance, which was uninfluenced by the albumin infusions.

Comment. The appreciable fraction of infused albumin still present 10 days after the infusions does not deviate from the

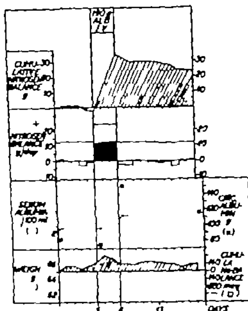


Fig. 1. Case 1. Metabolic data on patient with abnormal gastrointestinal protein loss before, during and after intravenous infusion of albumin. Nitrogen balance: The intake is plotted from baseline upwards, and urinary + fecal nitrogen is subtracted from this value. The distance between the net line and the zero indicates nitrogen balance. Above zero it is positive (black columns).

findings in normal persons (Eckhardt & Davidson 1950, Eckhardt, Lewis, Murphy, Batchelor & Davidson 1948). At this time the infused albumin has been evenly distributed in the total body pool of albumin (TA). Half of it had disappeared. This equals a degradation rate of about 7 % of TA per day $\left(\frac{\ln 2 \times 100}{10}\right)$.

The ^{131}I albumin degradation rate determined immediately after the infusion period amounted to 13.4 % of CA per day (table III), a high normal value[2].

[1] 25 % human serum albumin was supplied by Statens Seruminstitut, Copenhagen. Albumin nitrogen made up 98—99 % of total nitrogen.

[2] Normal range (mean ± 2 SD) of ^{131}I-albumin degradation is 5.6—14.4 % of CA per day (Jarnum & Schwartz 1960).

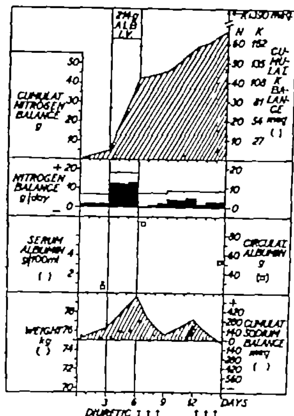


Fig 2. Case 2. Legend see fig 1

or about 6 % of TA, as CA at an average is 42 % of TA (Jarnum & Schwartz 1960) Thus there was a reasonably good agreement between the simultaneous elimination of infused albumin and ^{131}I albumin. None of them was abnormally high, which suggested that the patient's gastric protein loss was moderate at the time of the investigation.

Case 2 Male, 35 years. Giant hypertrophic gastritis.

The investigations were initiated a few days after he had been admitted severely debilitated with dyspnea, ascites and edema (on a rough estimate the extracellular fluid was increased by 15 liters) He was steadily improving throughout the investigations. This was reflected by the consistently positive nitrogen and potassium balance (fig 2) In 15 days he retained 66 g nitrogen and 390 mEq potassium.

Serum albumin and CA increased 3—4 times during the infusion period (table II) Nine days later they were still 50 and 100%, respectively above the preinfusion levels. The weight increased by 2 kg during the infusions parallel to a sodium retention of 280 mEq (= the sodium content of 2 liters of extracellular fluid) The administration of a diuretic (chlorothiazid, 1 g per day) released a prompt sodium loss with weight reduction.

Comment. The most remarkable finding in this patient was a marked retention of nitrogen and potassium. The nitrogen retention (66 g) equalled an increase of lean body mass by about 2 kg (Reifenstein, Albright & Wells 1945). The potassium retention (390 mEq) was partially due to a potassium deficiency at the beginning of the investigations, where serum potassium was slightly depressed. The cumulative potassium balance was twice as high as expected from the simultaneous nitrogen retention because the normal intracellular potassium/nitrogen — ratio (mEq/g) is about 2 7 (Reifenstein et al.)

The marked positive nitrogen balance was less pronounced immediately following the infusions. This suggested a breakdown of infused albumin. After the infusions CA decreased 0.73 g/kg in 8 days or 63 % of the increase produced by the infusions. Assuming an even distribution of infused albumin in the total body pool one may infer that about 40 % of the infused albumin was still present after 8 days. Thus

$$0.40 = 1 \times e^{-\lambda t}$$

where λ = fractional rate of total albumin degradation, and t = 8 days.

This gives a degradation rate of the total albumin pool of 11 % per day or about 26 % of CA per day if albumin distribution is normal (i. e. CA = 42 % of TA) ^{131}I-albumin metabolism was determined 7 months prior to the infusions at a time when serum albumin (1 1 g/

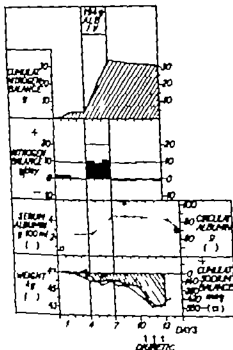


Fig. 3. Case 3. Legend see fig. 1

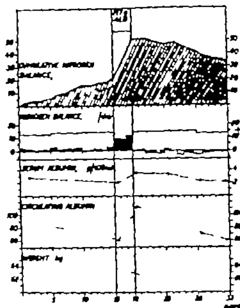


Fig. 4. Case 4. Legend see fig. 1

100 ml) was similar to the preinfusion level. The degradation rate was then 29 of CA per day

Case 3. Female, 28 years. Chronic exudative enteritis.

Before the infusions the nitrogen balance as slightly positive (fig. 3). Ninety-five per cent of infused albumin nitrogen was retained in the infusion period, while the cumulative nitrogen balance revealed loss of 3.4 g nitrogen (11 of the amount infused) in the following 6 days.

Two days after the infusions serum albumin and CA were twice as high as before the infusions. Six days later they had decreased by 52 and 60 respectively (table II). The weight did not decrease until diuretic as given. Sodium balance reflected the fluctuations of body weight.

Comment. Applying a calculation as described above it was found that about 12 % of infused albumin was degraded per day or 29 % of the intravascular fraction. The ^{131}I-albumin degradation was determined on two occasions 3 years before (when serum albumin and the state of edema were practically the same as before the infusions (Schwartz & Thomsen 1957)) and immediately following the infusions. The results were almost identical the degradation rat was 27.5 and 28.1 of CA per day respectively.

Case 4. Male, 38 years. Intestinal granulomatosis.

At the time of the investigations the patient was in recovery phase. His general condition was good, the weight increasing. He had slight edema and no ascites. Serum albumin was somewhat fluctuating (fig. 4).

The nitrogen balance in an initial period of 15 days was consistently positive. On an average he retained 10 % of the nitrogen supplied. In the infusion period he retained an amount of nitrogen equivalent to 83 of the albumin nitrogen, but after this a marked increase of urinary nitrogen was ob-

Table III Total retention of nitrogen and albumin during a 3 days period of albumin infusion together with the albumin degradation rate calculated from the elimination of infused albumin and ^{131}I-albumin

Patient No.	Nitrogen retention during infusion period, % of infused nitrogen	Estimated retention of infused albumin 1 day after infus. period %	Elimination as % of intravascular fraction per day of		Time of ^{131}I-albumin study
			Infused albumin	^{131}I-albumin	
1	120	96	17	13.4	Post-infusion period
2	107	80	26	29 0	7 months before infusion
3	95		29	27.5 28.1	3 years before infus. post-infusion period
4	83	59	36	29.3	Pre-infusion period

served which rendered his nitrogen balance almost consistently negative.

One day after the infusions serum albumin and CA had increased by 75 and 84 % respectively (table II) The rise decreased by 83 and 82 % respectively in 10 days, and after 14 days by almost 100 % The weight gradually increased by 2 5 kg in spite of a temporary weight reduction during the infusions. Sodium balance was slightly negative (5—10 mEq per day during the infusions 33 mEq per day)

Comment. The conversion of a positive nitrogen balance into a negative one following the infusions suggested a rapid degradation of infused albumin. The degradation rate was calculated to be 15 % per day or about 36 % of CA per day ^{131}I-albumin degradation rate determined in the initial period (15 days) before the infusions was 29 3 % of CA per day

Discussion and conclusion

The metabolic investigations described above revealed several common features in 4 patients with gastrointestinal protein loss

1 Three patients spontaneously displayed a *positive nitrogen balance* the fourth one (No. 1) whose gastric protein loss was moderate, maintained nitrogen equilibrium.

In spite of this, serum albumin remained low Consequently a protein-rich diet does not influence the hypoalbuminemia in these patients.

2. A marked positive nitrogen balance was observed during infusion of albumin (3—4 g per kg over a period of 3 days) It amounted to 83—120 % of the nitrogen infused (table III) *After the infusions the nitrogen balance was negative* in 3 and less positive in one (No 2)

The albumin infusions caused a pronounced increase of serum albumin and the amount of circulating albumin (CA) One day after the infusion period 60—100 % of the albumin was still present in the body After this it rapidly declined. Ten days later half (in No. 1) to 4/5 (in No. 4) of the albumin infused had been eliminated Thus it appeared that infusion of an amount of albumin equal to 2 times the normal CA (2 g per kg (Jarnum 1959 b)) could bring about only a transient increase of serum albumin concentration This invalidates the therapeutic benefit of albumin or plasma infusions in protein-losing gastroenteropathy

3. *A good agreement* was found *between* albumin degradation calculated from the *disappearance rate of infused albumin and the* ^{131}*I-albumin degradation rate*, whether the latter was determined immediately after the infusions (Nos. 1 and 3) or on a previous occasion (Nos. 2, 3 and 4). From this one would conclude

a) The endogenous *albumin production is not influenced by* a transitory more or less complete normalization of *the serum albumin concentration*.

b) The *relative albumin breakdown* ("fractional rate of turnover") is *independent of the serum albumin concentration*, or — in other words — the amount of albumin degraded (g per day) is proportional to serum albumin concentration.

In this respect the process by which an abnormal amount of albumin is transferred to the gastrointestinal tract, is similar to the renal excretion of a pure filtration substance (e. g. inulin). At the same time it seems to indicate that the basic pathogenic lesion is not one of an abnormal secretion of plasma proteins. If this were the case one would expect a "depression" of the protein loss at high serum albumin levels.

Summary

Metabolic investigations were carried out before during and after intravenous infusion of albumin (3—4 g per kg) in 4 patients with abnormal gastrointestinal protein loss.

1. Before the infusions the patients maintained nitrogen equilibrium or retained nitrogen.

2. A pronounced nitrogen retention was observed during the infusions.

3. After the infusions 3 patients displayed a negative nitrogen balance indicating a rapid breakdown of infused albumin. Ten days after the infusions 50 to 80 % of infused albumin was eliminated.

4. Albumin degradation calculated from the disappearance rate of infused albumin agreed well with ^{131}I-albumin degradation values in the same patients.

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Determination of the Triiodothyronine Uptake by Erythrocytes — a Practical Routine Test of Thyroid Function[1]

By

Anders Parrow and Ivar Werner

Disorders of the thyroid constitute a clinically important group of diseases. Most of them have long been successfully treated and the patients restored to perfectly normal health provided a correct diagnosis is established within reasonable time. Consequently the diagnostic problems of thyroid disease have been studied intensely. Usually careful clinical examination will give a correct diagnosis in cases of hyper- as well as of hypothyroidism, and no laboratory method will replace it in the everyday dealing with patients. Yet it must be admitted that on many occasions the decisions have to be based more or less exclusively on the laboratory tests. This is often the case for example in hyperthyroidism in old age, or in menopausal disturbances simulating hyper- or hypothyroidism in euthyroid women.

The laboratory methods most extensively used today are the BMR, the radioiodine uptake and excretion, and the PBI. All of them have advantages and disadvantages. The oldest and simplest method is the BMR-determination. It does not require expensive equipment and can be repeated as often as wished. The drawback is, however, that it measures the oxygen consumption without giving information about the cause of an increase or a decrease. The BMR is thus a very unspecific indicator of the functional state of the thyroid gland.

The radioiodine test, which measures phases of the iodine metabolism in the thyroid gland, more specifically depicts the functional state of the thyroid. However, in addition to the elaborate experimental set-up often required, it has two main disadvantages. The test cannot be repeated whenever desired and there is not always a clear distinction between

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[1] Part of this work has been reported to the Swedish Endocrine Society in 1958 and 1959.

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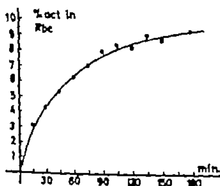


Fig. 1. Influence of incubation time on the rbc uptake of TIT

blood samples is avoided. For the determination an International Micro-Capillary centrifuge, Model MB, has been used. The hematocrit includes the volume of the white blood cells.

The blood samples have not routinely been taken under sterile conditions. For that reason they have been stored at + 4 C until used. Experiments have shown that blood treated in this way can be kept for at least one week without change in the RCU. When the blood is stored at room temperature the RCU will increase significantly even within 24 hours and this increase will continue during the following days. Blood collected under sterile conditions does not show any such increase in RCU when stored at room temperature for four days.

Other experiments have shown that the RCU will not be altered if the rbc are suspended in serum instead of plasma.

Incubation

The RCU has been determined in duplicate for each blood sample. Two 3-ml aliquots of blood were transferred to 10 ml Erlenmeyer flasks and the I[131]-labelled TIT in 0.1 ml of saline was added.

As has been stated by Hamolsky et al. heparin can be used just as well as potassium oxalate. It must be pointed out, however, that the hematocrit value of the heparinized blood will be about 10 higher than that of the oxalate blood. That, of course, is due to the difference in osmotic characteristics.

I[131]-labelled TIT was purchased from Abbott Lab. Oak Ridge, Tennessee, U.S.A.

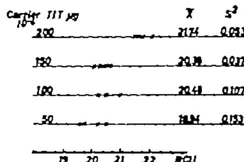


Fig. 2. Influence on RCU of varying amounts of carrier TIT. As seen there are no significant differences among the first three series. If, however, a F-test is applied on the assumption $\mu I = \mu II = \mu III = \mu IV$ clearly significant figure is obtained (F = 34.2 D. fr = 3 and 16 respectively). This seems to be solely due to deviation of series IV.

After the addition of TIT the stoppered flasks were shaken continuously and vigorously for 2 hours in a thermostated room t + 37° C. As shown by Ureles et al. and Hamolsky et al., it is important that the flasks be stoppered, otherwise the change in CO_2 tension in the blood will result in a significant decrease in RCU (20, 7). The RCU increases with increasing incubation time and equilibrium is not reached even after 3 hours (fig. 1). This is in accordance with earlier reports (6, 4). As is seen in fig. 1 the increase in RCU per minute after 2 hours of incubation is small and an incubation time of 2 hours ± 5 minutes will give acceptable reproducibility. In this investigation the variation of incubation time has been kept at ± minutes.

A series of experiments was undertaken in order to find out if variations in the amount of TIT added would influence the RCU. As seen in fig. 2 the RCU increases about 10 per cent when the amount of TIT is increased from 50×10^{-4} microg/3 ml of blood to 200×10^{-4}. Although this increase is rather small it cannot be considered negligible. Thus it is desirable to keep the added amount of TIT within narrow limits, and as routine we have used 80—120 $\times 10^{-4}$ microg.

The TIT solution is rather quickly destroyed at room temperature but stored at + 4 C it will not be broken down to a

normal and abnormal values. Furthermore, the results are influenced by factors such as the iodine intake prevailing previous to the test.

The serum level of PBI (or the butanol-extractable iodine, BEI) also usually gives a good measure of the state of thyroid function. The methods of determination are laborious and need careful and skilled handling however Further the results are significantly affected by previous iodine administration.

Thus all the tests have limitations which should render further search for new laboratory aids most desirable. In 1957 Hamolsky et al. published a report on a new thyroid function test which seemed to offer many advantages (6) They showed that the *in vitro* uptake of I^{131}-labelled *l*-triiodothyronine (TIT) by human erythrocytes, RCU (= red cell uptake) was closely correlated with thyroid function The RCU was increased in blood from hyperthyroid individuals and decreased in blood from hypothyroids Their results further indicated that the RCU was determined by the plasma milieu and not by the red cells themselves, *i e* the RCU is the same in the same plasma whatever may be the origin of the erythrocytes. There was very little overlapping between groups of patients with different types of thyroid function *i e* the degree of diagnostic exactness seemed to be satisfactory The results were not influenced by hypermetabolism of non-thyroidal origin or by iodine administration in moderate dosage. On the other hand the RCU was elevated in some euthyroid states with changes in the plasma protein pattern such as nephrosis, and in some cases of malignant disease. A decreased RCU was regularly found in pregnancy

The present investigation was begun in 1958 in order to evaluate the test as a routine method for the assay of thyroid function. During the course of the investigation several new reports on the subject have appeared (3, 4 7 9, 13 16 17 18, 19 20 21) The results of Hamolsky et al. have for the most part been confirmed. In most reports, however the overlapping between normal and pathological groups has been greater than in Hamolsky's original study The levels of the RCU values have also differed in several studies. As our preliminary results showed considerably higher RCU values than those reported by Hamolsky et al. and also some other differences, the investigation was extended to include a study of methodological details in order to explain the variation in results among different laboratories.

I. Methodological study

Mainly the method described by Hamolsky et al. has been used in this investigation. I^{131}-labelled TIT is added to oxalate venous blood and the mixture incubated at 37° C. The radioactivity is then determined in whole blood and in the rbc. The rbc activity is calculated as per cent of the total activity The RCU value given is the rbc uptake corrected to a theoretical hematocrit of 100 %.

The collection of blood

In the same manner as Hamolsky et al., we have throughout our study used 0.1 ml of a saturated potassium oxalate solution in 10 ml of blood drawn from both fasting or non-fasting patients. It has not been possible to draw exact amounts of blood, but experiments have shown that a variation between 8 and 12 ml of blood to 0.1 ml of oxalate solution does not significantly influence the RCU The accuracy which is obtained using ungraded 12 ml centrifuge tubes is thus sufficient.

The hematocrit and the RCU have been determined on the same blood sample In this way the error which may be caused by variations in hematocrit between two different

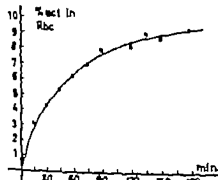


Fig. 1 Influence of incubation time on the rbc uptake of TIT

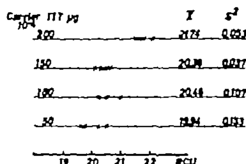


Fig. 2. Influence on RCU of varying amounts of carrier TIT As seen there are no significant differences among the first three series. If, however F-test is applied on the assumption $\mu_I = \mu_{II} = \mu_{III} = \mu_{IV}$ clearly significant figure is obtained (F = 34.2; D fr = 3 and 16 respectively). This seems to be solely due to deviation of series IV

blood samples is avoided. For the determination an International Micro-Capillary centrifuge Model MB, has been used. The hematocrit includes the volume of the white blood cells.

The blood samples have not routinely been taken under sterile conditions. For that reason they have been stored at + 4 C until used. Experiments have shown that blood treated in this way can be kept for t least one week ithout change in the RCU When the blood is stored at room temperature the RCU will increase significantly even within 24 hours and this increase will continue during the following days. Blood collected under sterile conditions does not show any such increase in RCU when stored at room temperature for four days.

Other experiments hav shown that the RCU will not be altered if the rbc are suspended in serum instead of plasma.

Incubation

The RCU has been determined in duplicate for each blood sample. Two 3-ml aliquots of blood were transferred to 10 ml Erlenmeyer flasks and the I¹³¹-labelled TIT in 0.1 ml of saline was added.

As has been stated by Hamolsky et al. heparin can be used just as well as potassium oxalate. It must be pointed out, however that the hematocrit value of the heparinized blood will be about 10 higher than that of the oxalate blood. That, of course, is due to the difference in osmotic characteristics.

I¹³¹-labelled TIT was purchased from Abbot Lab., Oak Ridge Tennessee, U S. A.

After the addition of TIT the stoppered flasks were shaken continuously and vigorously for 2 hours in a thermostated room at + 37° C. As shown by Uredes et al. and Hamolsky et al., it is important that the flasks be stoppered, otherwise the change in CO tension in the blood will result in a significant decrease in RCU (20, 7) The RCU increases with increasing incubation time and equilibrium is not reached even after 3 hours (fig 1) This is in accordance with earlier reports (6 4) As is seen in fig. 1 the increase in RCU per minute after 2 hours of incubation is small and an incubation time of 2 hours ± 5 minutes will give acceptable reproducibility In this investigation the variation of incubation time has been kept at ± 2 minutes.

A series of experiments was undertaken in order to find out if variations in the amount of TIT added would influence the RCU As seen in fig. 2 the RCU increases about 10 per cent when the amount of TIT is increased from 50×10^{-4} microg/3 ml of blood to 200×10^{-4} Although this increase is rather small it cannot be considered negligible. Thus it is desirable to keep the added amount of TIT within narrow limits, and as a routine we hav used $80-120 \times 10^{-4}$ microg

The TIT solution is rather quickly destroyed t room temperature but stored t + 4 C it will not be broken down so

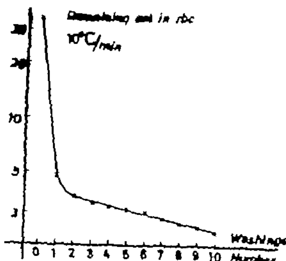


Fig 5 Influence of the number of washings on the activity remaining in the rbc. Note the linear disappearance rate after second washing corresponding to a loss of about five per cent of the remaining activity in each washing

degree which will influence the RCU On account of the 8-day half-life of I[131] the solution has to be renewed once a month, otherwise the low activity will necessitate a very long counting time.

Preparation of the rbc sample

From each of the two Erlenmeyer flasks 1 ml of blood was transferred in duplicate into polyethylene tubes. In one tube the whole blood activity was determined. The blood in the other was immediately mixed with 5 ml of sterile saline at room temperature. The tube was then centrifuged for 3 minutes at 2,500 rpm and the supernatant removed by suction through a capillary The rbc were thereupon washed four times in the tube with 5 ml of saline each, which was added under pressure from a syringe.

Each washing diminishes the activity in the tube The loss of activity is greatest in the first washing when most of the plasma is removed. In the following washings the percentage loss of activity is fairly constant, even if repeated 10 times (fig 5) When the activity remaining in the tube is plotted against the number of washings in a semilogarithmic system the points will form a straight line. This agrees well with the result of other investigations and illustrates the importance of keeping the number of washings constant (12, 1)

In another series of experiments the tubes were kept for times varying from 5 to 60 minutes at room temperature after the incubation and before the start of the washings. It was found that with increasing "waiting" time the activity remaining in rbc decreased. The decrease in RCU during the first 15 to 20 minutes was hardly significant but thereafter the value diminished about 10 to 15 %. It is thus evident that the whole washing procedure must be standardized.

In their first report Hamolsky et al. also mentioned that the RCU can be determined by measuring the activity remaining in plasma after incubation. In order to do this a small amount of plasma was removed from the centrifuged blood sample. The total activity in the plasma was then calculated and subtracted from the whole blood activity For practical purposes this would be a simpler method but our RCU values obtained in this way were very unreliable. The reason for this may be twofold. In the first place the error will be greater when a small value is determined as a difference between two high values. Secondly the activity of the plasma sample lost by adsorption to the glassware will be greater as the plasma will be pipetted one time more than the whole blood sample.

Counting and calculation

Each tube was counted in a well-type scintillation counter and the counting time has been chosen to give an error of ± 1—2 RCU is then calculated as the rbc uptake at the theoretical hematocrit 100 %, by the formula

$$RCU = \frac{\text{rbc act.} \times 100 \times 100}{\text{whole blood act.} \times \text{hct}}$$

As mentioned by Hamolsky et al. the correction to hct 100 is not quite satisfactory In the same plasma-rbc system the RCU determined at a low hematocrit will be lower than when determined at a high hematocrit. This has also been shown by other investigators (1 9) In the present investigation this error is not further considered. Special studies concerning this problem are being conducted, which will be published later (14)

Error of the method

The error of the method calculated as standard error of the differences between the double determinations in the group of euthyroid females (134 cases) was ± 0.44 (standard error = ± $\sqrt{\frac{\Sigma d^2}{2N}}$). The standard error thus obtained cannot, however be measure of the total error of the method. The difference between the duplicate determinations does not include the errors which may result from the handling of the blood sample before its being divided in the two Erlenmeyer flasks. This has been made the subject of a later investigation (15) The error of the method was then calculated from different materials in which RCU was determined in the usual way from two 10 ml blood samples instead of one. It was found that the total error of the method (S) calculated from the differences between the two blood samples was of bout the same size as the error of the method obtained from the differences between the ordinary double determinations ( ) From this and other control experiments it seems reasonable to assume that S in the present material probably does not exceed ± 0.50. For practical purposes it might be concluded that the total error of the method will not exceed ± 1.5 when the blood is drawn by persons not specially trained in this method. I method using radioactive isotopes there is always considerable risk of accidental error due to contaminated tubes and glassware In this special method there is further risk of error due to loss of the during the washings. These errors usually will be shown by an increase in the difference between the double determinations I our experiments when this difference has exceeded 10 per cent of the mean value the determination has been regarded as failure

II Clinical study

Material

Euthyroid For this study these were divided into two groups. One group, 213 persons (134 females, 79 males), was classified as "normal" euthyroids and consisted of healthy medical students and of patients suffering from cardiac disease minor psychic disorders, obesity bronchial asthma and acute infectious disease in whom careful clinical and laboratory investigation had revealed no sign of thyroid abnormality

The other group, 49 persons (30 females, 19 males) comprised patients suffering from nephrosis, liver cirrhosis, metastatic malignancy myelomatosis, uremia, long-standing severe infectious disease, leukemia, or polycythemia. There were also 15 pregnant women in that group.

Hyperthyroid 54 patients (45 females, 9 males) with untreated hyperthyroidism were studied. 36 of these had toxic diffuse goiter TDG and 18 toxic nodular goiter TNG The diagnostic differentiation was based on histological examination after surgery Ten further cases were also studied during the course of treatment.

Hypothyroid 15 patients (12 women, 3 men) with untreated myxedema were studied. Ten of these cases were also studied during treatment.

All patients had been admitted to the medical and/or surgical departments of the University Hospital between June 1958 and June 1959 The diagnosis hyper and hypothyroidism was based on ordinary clinical and laboratory criteria such as BMR, PBI and I^{131}-test and has in each case been confirmed by the results of treatment.

In many cases the RCU was determined in the same person more than once. In this report only the first determination is listed. In this way better information as to the diagnostic value of the test will be obtained and the risk of a biased selection of the material is avoided.

Results

Euthyroids

Normal men The normal range was found to be 14 7—26.0 %, with a mean value of 20.2 ± 0.28, standard deviation 2.52. Out of the 79 men 4 had an RCU above 25.0 and one below 15.0 %

Normal women The normal range was found to be 12 1—27 1 mean value 18.9 ± 0.22, standard deviation 2.55 Out of 134 women 4 had an RCU higher than 4.0 and 7 below 14.0 %

Table I The RCU distribution in euthyroid subjects and untreated hyper and hypothyroid subjects

	N	RCU	M	s
A. Euthyroids				
Females	123	14.0–24.0		
	7	< 14.0		
	4	> 24.0		
	134	12.1–27 1	18.9 ± 0.22	2.55
Males	74	15.0–25.0		
	4	> 25.0		
	1	< 15.0		
	79	14.7–26.0	20.2 ± 0.28	2.52
Total	213	12.1–27 1	19.0 ± 0.18	2.66
B. Toxic diffuse goiter (TDG)				
Females	27	> 24.0		
	2	< 24.0		
Males	5	> 25.0		
	2	< 25.0		
Total	36	20.3–38.1	28.9 ± 0.75	4.49
C. Toxi nodular goiter (TNG)				
Females	3	> 24 0		
	13	< 24 0		
Males	1	> 25.0		
	1	< 25.0		
Total	18	16.6–36.6	23.3 ± 1.28	5.43
D Myxedema				
Females	10	< 14 0		
	2	> 14.0		
Males	3	< 15.0		
Total	15	7.8–15.2	12.5 ± 0.51	1.96

The difference between the mean values for men and women is statistically highly significant. The total data are given in table I

Miscellaneous diseases An elevated RCU was found in some, but not all euthyroid patients suffering from the following diseases nephrosis, uremia, cirrhosis of the liver leukemia, polycythemia vera, myelomatosis, severe infectious diseases of long standing *e g* abdominal and pulmonary abscesses, staphylococcal septichemia, metastatic malignancies. The elevated values are listed in table II

Table II RCU values in some cases of non-thyroid disorders. All determinations performed after correction of hematocrit to values around 40 %

Case	Diagnosis	RCU
S. M ♀ 57	Pulmonary abscess	27.8
E. S. ♂ 52	Abdominal abscess	26.8
H. K. ♂ 67	Myelomatosis.	26.1
B. E. ♂ 56	Nephrosis	26.3
W Å. ♂ 58	Nephrosis	27.5
W G ♂ 53	Uremia	27.0
G. T ♀ 76	Uremia	35.4
L. V ♀ 78	Acute leukemia	32.3
H. M. ♀ 63	Polycythemia vera	29.2
J K. ♀ 65	Polycythemia vera	28.4
H. K. ♀ 54	Metastatic malignancy	38.4
L. E. ♂ 66	Metastatic malignancy	32.3

A low RCU has regularly been found in pregnant euthyroid women. All of them had an RCU below 12.5 %

Hyperthyroids

No subdivision into men and women has been made in this study on account of the small number of males.

TDG (29 women, 7 men) The range was 20.3—38.1 % mean value 28.9 ± 0 75 standard deviation 4 49 Two men had an RCU below 25 0 % and two women had an RCU below 24 0 %. Thus 4 patients out of 36 had an RCU within the upper normal limit. The difference between the mean values of TDG and euthyroids is statistically highly significant (table I)

TNG (16 women and 2 men) The range was 16 6—36 6 % mean value 23.3 ± 1.28 and standard deviation 5.43

Table III. Change in RCU during preoperative iodine treatment

Case	RCU		Duration of therapy days
	Before therapy	After therapy	
E. A. ♀ 74	25.5	17.4	9
J. M. ♀ 29	28.4	23.3	9
W. S. ♀ 32	23.4	20.3	10
W. L. ♀ 56	32.6	17.9	12
W. K. ♂ 43	24.1	19.9	14

Table IV. The effect of thyrostatic therapy on RCU in hyperthyroidism

Case	RCU		Therapy
	Before therapy	After therapy	
O. A. ♀ 63	28.0	22.5	PTU 2 weeks
J.S.G. ♂ 58	26.0	21.6	PTU 4 weeks
P. M. ♀ 64	30.0	23.5	PTU 4 weeks
P. M. ♀ 64	30.0	21.0	PTU 16 weeks
L. R. ♀ 18	40.0	19.6	MMI 5 weeks
H. V. ♀ 73	34.7	24.2	PTU 6 weeks
J. E. ♂ 55	29.6	23.7	PTU 6 weeks
J. M. ♀ 15	31.2	21.8	PTU 8 weeks
K. K. ♀ 66	23.7	18.2	EMI 8 weeks
A. A. ♀ 27	30.2	19.9	MMI 16 weeks
J. O. ♂ 27	28.0	20.4	PTU 6 months

PTU = propylthiouracil.
MMI = merkaptomethylimidazol.
EMI = ethoxycarbonylthiomethylimidazol.

Table V. Effect on RCU of partial thyroidectomy

Case	RCU		Time after operation
	Before therapy	After therapy	
B. J. ♀ 60	32.5	22.2	1 week
S. A. ♀ 50	38.1	18.9	1 week
J. L. ♀ 25	29.4	18.8	2 weeks
B. H. ♀ 75	27.5	17.1	4 months
H. A. ♀ 50	31.5	18.5	6 months

Table VI. RCU before and during treatment of hypothyroidism

Case	RCU		Duration of therapy and metabolic state
	Before therapy	During therapy	
E. G. ♂ 29	13.4	17.5	5 weeks, not compensated
A. E. ♀ 55	10.1	16.1	6 weeks, not compensated
R. L. x 62	12.7	17.6	8 weeks, not compensated
L. L. ♀ 60	13.4	15.3	6 months, compensated
A. B. ♂ 40	12.5	15.5	7 months, compensated
F. E. ♀ 55	11.9	18.8	12 months, compensated
W. I. 55	12.9	20.5	12 months, compensated

Three women had an RCU above 24.0 %. One man had an RCU above 23.0 %. All the others had an RCU well within the normal range. However the mean value of the group differs significantly from the mean value of the euthyroids as well as from that of the TDG group.

Effect of therapy. Iodine treatment decreased the RCU if given in amounts sufficient to decrease the metabolism as for instance in preoperative treatment. The effect on the RCU followed the metabolism fairly well and a normal RCU was as a rule found when the patient was euthyroid as determined by bedside examination. In one case of hyperthyroidism the RCU was normalized following 2 weeks of iodine treatment after which time for certain reasons the administration was stopped. In another fortnight the patient was back in the

hyperthyroid state as verified by BMR and bedside examination. The RCU was again strongly elevated The data from some typical cases are given in table III

Thyrostatic remedies also decreased the high RCU This decrease followed the decrease in metabolism very well Examples are given in table IV

After successful surgical treatment the RCU has been restored to normal and remained so in all cases followed (20) except one. (Some typical cases are listed in table V) In this one patient the RCU one year after operation was still 30 1 % in spite of the fact that the patient had a myxedematous appearance with a BMR of — 22 %

In a few instances we have noted a decrease of RCU in hyperthyroid patients treated only with barbiturates.

Hypothyroids

The range was found to be 7 8—15 2 % mean 12.5 ± 0.51 standard deviation 1 96 The 3 men in this group all had an RCU below 15 0 % and of the 12 women only 2 had an RCU above 14 0 % The difference between the mean values of the hypothyroid group and the euthyroid group is statistically highly significant (table I)

Effect of therapy Substitution therapy in myxedema increased the RCU in all hypothyroid patients as illustrated in table VI As seen an increase of RCU to a normal value does not necessarily mean that the hypometabolic state is fully compensated

Discussion

The results of this investigation essentially agree with earlier reports on RCU Thus they confirm the findings of an elevated RCU in hyperthyroidism and lowered values in hypothyroidism

Our uptake values are considerably higher than those earlier reported. Hamolsky et al found the following normal values Men 11 8—19 0 % mean 15.3 % women 11 0—17 0 % mean 13.9 %. Values in agreement with Hamolsky's were found by Ureles & Murray Norm, Young et al. (20 13 21) and somewhat higher values were reported by Sharpe and Robbins 10 1—23 0 % (18 16) Schumacher et al have found much higher values but i. a. they determine the RCU after 3 hours incubation (17) The cause of these differences is not quite clear Several explanations may be considered.

There may for example be geographical differences in the RCU levels, as is the case for instance with the radioiodine-uptake (cf. Linderholm & Werner) (8)

More probable perhaps is that differences in the method will explain the different RCU levels. It is clearly shown in this study and also by Adams et al. (1) that the number of washings is of importance for the result Meade has further shown that the time during which the rbc are suspended in saline during washing is of importance as is the saline volume in each washing (11) Marks et al. could not find any influence on RCU when the saline volume was increased 3 times (9) Our own experience agrees essentially with that of Marks. A detailed report on the influence of the saline volume will be published later (14 b)

As is shown in the methodological study the time interval between the incubation at 37 C and the start of the washings is another factor of importance. The longer the interval the lower the RCU It seems probable that different laboratories may have different ways of handling the samples in this respect

Further the influence of the hematocrit must be considered. The addition of 0.1 ml of saturated oxalate solution to 10 ml of blood will result in a hematocrit about 10 % lower than that of the same blood drawn into heparin. The mean RCU value of normal euthyroid men in this investigation is 20.2 %. The mean hematocrit value in the group is 39.8 % In heparin blood the absolute uptake is the same as in oxalate blood (from our own experiments) It means that the mean RCU 20.2 % corresponds to 18.5 in heparin blood in which the hematocrit would be about 44 %. This hematocrit effect may explain part of the difference in RCU between different materials

It is also possible that the degree of shaking during incubation is of some importance A too intense shaking may partly denaturate the TIT binding prote in as is the case e g with some of the serum protein complements. Destruc tion of the TIT-binding protein results in an increased RCU (14)

In earlier investigations little is men tioned about the frequency of toxic nodular goiter TNG Lreles et al found 2 cases of TNG among 26 hyperthyroid patients and these two had an elevated RCU in spite of having a normal PBI In our material about 30 of the hyperthyroid patients were TNG Only few of the patients had an elevated RCU It is extremely unlikely that the generally low RCU values in this group could be the result of a chance gathering as the difference between the values of TDG and TNG is statistically significant. Clinically TDG and TNG often behave differently in many respects. It is also well know that laboratory diagnostic aids are of less value in cases of TNG than in TDG. Thus McConahey & Clarke found that the radioiodine test often will give normal values in cases of TNG (10) Of interest is also the investigation of Hamolsky and Freedberg (5) They studied in dogs the disappearance rate from the circulation of thyroxine injected together with serum from patients differing in thyroid function. The half-life of the hormone was significantly shorter when it was injected together with serum from patients with TDG than when injected with serum from euthyroid patients. When serum from patients with TNG was used the half life was not shortened. Thus many facts support the view that TDG and TNG although closely related, may not be metabolically identical. The difference in RCU observed is probably another expression of this dissimilarity

The diagnosis of TNG in our material is based upon the findings at operation and cases with one or several adenomas in the gland have been classified as TNG This does not necessarily mean that the adenoma has been the cause of the hyper function. It seems likely that even an adenomatous gland may be subject to diffuse hyperfunction. It is also reasonable to assume that some adenomas will behave more or less exactly like a TDG Thus it is not astonishing to find some patients with TNG who behave like patients with TDG

In agreement with Hamolsky et l. we have also found a low RCU in pregnancy and high values in euthyroids suffering from diseases accompanied by profound plasma protein changes such as metastatic malignancy myelomatosis leukemia, cirrhosis of the liver nephrosis etc. In this connection the leukemias deserve special comment. The high uptake in cases of leukemia could be due to the increased amounts of leukocytes and thus due to a mechanism different

from that assumed for the other diseases mentioned. However in experiments we have found the uptake in leukocytes to be of the same order of magnitude as in erythrocytes. In wbc from patients with an acute blastic leukemia the uptake is sometimes more than twice that of the rbc, but even this increased ability to take up TIT will not be enough to explain the increased RCU in these patients

The mechanism underlying the RCU is not yet clear As stated by Hamolsky et al. (1959) the simplest explanation is that the RCU is a "spilling over" effect. This means that the rbc are able to take up only that part of the added hormone which the plasma proteins cannot bind. Several investigations have shown that the plasma proteins have an ability to bind thyroid hormone. It has also been shown that the plasma proteins can be saturated when increasing amounts of hormone is added to the blood. Christensen has shown that the RCU is correlated with the amount of free diffusible thyroxine, which also supports the "spilling over" theory (2) This problem is the subject of further investigation which will be published later together with a detailed discussion (14 a)

As the number of RCU determinations in the hyper- and hypothyroid groups are fairly small, the RCU variation expressed in terms of ± 2 standard deviations is not of much help in determining the border lines between normal and pathological states. Regarding the hyperthyroid group TDG this is further complicated by a probably skew distribution of the values If the normal values for men are set to 15.0—25 0 % and for women to 14 0—24 0 % about 4 % of the "normals" had an RCU value above the upper limit and about 4 % a value below the lower limit. Only 4 of the TDG[1] and 2 hypothyroids fell within the normal limits Thus these limits seem to serve practical purposes fairly well.

It may be concluded that the determination of RCU is a valuable screening test for thyroid function. It is fairly simple, it is inexpensive, it is not influenced by iodine administration in moderate dosage, is not influenced by fever or exercise and other factors which render the BMR unreliable, and since it is performed *in vitro* the patient will not be exposed to radioactivity and therefore the test can be repeated whenever desired. Thus it can be considered as a good adjunct to other thyroid function tests in spite of the fact that it is not a specific parameter of thyroid function. It is of special value in the diagnosis of hyperthyroidism due to toxic diffuse goiter

Summary

The *in vitro* uptake of I[131]-labelled *l*-triiodothyronine (TIT) by human erythrocytes (RCU) in different states of thyroid function was first described by Hamolsky et al. who found that RCU usually was correlated with thyroid function. The present paper reports a study of the reproducibility of the RCU determinations and their use as a clinical routine test.

The reproducibility of the RCU determinations is highly dependent on a strict standardization of the laboratory procedure Especially important are the amount of TIT added to the blood, the time of incubation of the TIT-blood mixture the time interval between the end of the incubation and the start of washing and the number of washings.

[1] Among these 4 patients the diagnosis hyperthyroidism was rather uncertain in 3.

The RCU normal values obtained by our standardized" procedure are 15—25 per cent in males and 14—24 per cent in females. These values are considerably higher than those reported by Hamolsky et al. The difference in mean values of males and females is statistically significant.

The RCU mean value is significantly lower in untreated hypothyroidism, and significantly higher in untreated hyperthyroidism than in the euthyroid state. This agrees with the results reported by Hamolsky et al. and other authors.

Patients with toxic diffuse goiter have a significantly higher RCU mean value than patients with toxic nodular goiter among whom normal RCU values are frequently found.

In agreement with earlier reports, a high RCU is often found in euthyroid patients suffering from non-thyroidal diseases, especially those characterized by plasma protein abnormalities. Low RCU is regularly found among pregnant women.

RCU quite closely reflects changes in the metabolic state in hypo- as well as hyperthyroidism and is thus influenced by therapy.

The method is rather inexpensive and simple, and has many advantages over other methods. Thus it can be considered of great value as a screening test for thyroid function.

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The mechanism underlying the RCU is not yet clear As stated by Hamolsky et al (1959) the simplest explanation is that the RCU is a spilling over' effect. This means that the rbc are able to take up only that part of the added hormone which the plasma proteins cannot bind Several investigations have shown that the plasma proteins have an ability to bind thyroid hormone. It has also been shown that the plasma proteins can be saturated when increasing amounts of hormone is added to the blood. Christensen has shown that the RCU is correlated with the amount of free diffusible thyroxine, which also supports the "spilling over theory (2) This problem is the subject of further investigation which will be published later together with a detailed discussion (14 a)

As the number of RCU determinations in the hyper and hypothyroid groups are fairly small, the RCU variation expressed in terms of ± 2 standard deviations is not of much help in determining the border lines between normal and pathological states. Regarding the hyperthyroid group TDG this is further complicated by a probably skew distribution of the values. If the normal values for men are set to 15 0—25.0 % and for women to 14 0—24 0 / about 4 % of the "normals had an RCU value above the upper limit, and about 4 / a value below the lower limit. Only 4 of the TDG[1] and 2 hypothyroids fell within the normal limits. Thus these limits seem to serve practical purposes fairly well.

It may be concluded that the determination of RCU is a valuable screening test for thyroid function. It is fairly simple, it is inexpensive, it is not influenced by iodine administration in moderate dosage, is not influenced by fever or exercise and other factors which render the BMR unreliable, and since it is performed *in vitro* the patient will not be exposed to radioactivity and therefore the test can be repeated whenever desired. Thus it can be considered as a good adjunct to other thyroid function tests in spite of the fact that it is not a specific parameter of thyroid function. It is of special value in the diagnosis of hyperthyroidism due to toxic diffuse goiter

Summary

The *in vitro* uptake of I^{131}-labelled *l* triiodothyronine (TIT) by human erythrocytes (RCU) in different states of thyroid function was first described by Hamolsky et al. who found that RCU usually was correlated with thyroid function The present paper reports a study of the reproducibility of the RCU determinations and their use as a clinical routine test.

The reproducibility of the RCU determinations is highly dependent on a strict standardization of the laboratory procedure. Especially important are the amount of TIT added to the blood the time of incubation of the TIT blood mixture the time interval between the end of the incubation and the start of washing and the number of washings.

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The RCU normal values obtained by our "standardized" procedure are 15—25 per cent in males and 14—24 per cent in females. These values are considerably higher than those reported by Hamolsky et al. The difference in mean values of males and females is statistically significant.

The RCU mean value is significantly lower in untreated hypothyroidism, and significantly higher in untreated hyperthyroidism than in the euthyroid state. This agrees with the results reported by Hamolsky et al. and other authors.

Patients with toxic diffuse goiter have a significantly higher RCU mean value than patients with toxic nodular goiter among whom normal RCU values are frequently found.

In agreement with earlier reports, a high RCU is often found in euthyroid patients suffering from non-thyroidal diseases, especially those characterized by plasma protein abnormalities. Low RCU is regularly found among pregnant women.

RCU quite closely reflects changes in the metabolic state in hypo- as well as hyperthyroidism and is thus influenced by therapy.

The method is rather inexpensive and simple, and has many advantages over other methods. Thus it can be considered of great value as a screening test for thyroid function.

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Effect of Dicoumarol on the Transaminase Activity in Normal Persons

By

JØRGEN LYNGBYE

During the last few years reports have been published of higher concentrations of serum glutamic-oxaloacetic transaminase (SGO-T) and, especially of serum glutamic pyruvic transaminase (SGP T) after administration of certain drugs such as anticoagulants after short (1) or long-term treatment (2) supposedly due to a toxic effect on the liver. In such cases it was stated to be typical for SGP T to rise to higher values than SGO-T (1).

These circumstances would make it more difficult to evaluate transaminase determinations in myocardial infarction, as a secondary transaminase increase caused by dicoumarol might be misinterpreted as indicating an extension of the existing infarction or a new one.

A transaminase increase after administration of dicoumarol is stated to be particularly pronounced in cases with stasis of the liver following congestive cardiac failure.

The material available is scarce and a more thorough investigation of the problem would seem desirable. For this purpose the effect of dicoumarol on SGO-T and SGP T was examined in ten normal persons and four with advanced stasis of the liver.

Methods and material

On the first day of the experimental period at 8 a. m. one dose of 500 mg dicoumarol was administered orally to all patients except one, No. 12, who received two doses of 500 mg dicoumarol each at an interval of 24 hours. Immediately before and during four to five days after the administration determinations were made daily of SGO-T, SGP T (3), and the prothrombin index (4).

Experiments 1—10 were made in normal persons, experiments 11—13 in patients suffering from severe mitral valvular disease and stasis-conditioned enlargement of the liver, while experiment No. 14 was made in a woman of 55 with respiratory insufficiency on account of pulmonary emphysema and chronic bronchitis, this case also showed considerable stasis of the liver.

As far as possible the patients received no other drugs during the experimental period, and substances known to affect the transaminase values were in any case avoided.

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Results and discussion

Results are shown in table I. In patients 1—13 no or only a slight increase of either of the transaminase values was observed, no case rising to pathological values and, in particular, no increase of SGP-T. Patient No. 11, who suffered from very severe stasis of the liver, is especially significant. Only patient No. 14 showed a pathological increase of both of the transaminase values. In this case the increase of the transaminase values coincided with a clinical aggravation of the condition which judging from symptoms and electrocardiographic findings presumably was due to pulmonary embolism or thrombosis.

It will be seen that administration of dicoumarol in a single dose 2 1/2 times larger than that used by Wroblewski & Manso produced no or only a slight rise in the transaminase concentrations. A change of the SGO-T values of the order of magnitude found here will not give rise to diagnostic misinterpretations. In acute infarction and extension of an infarct the rise will be much greater. During the time when determinations of serum transaminases have been made on all patients in the ward who suffered from or were suspected of acute cardiac infarction, in most cases treated with dicoumarol there have been no diagnostic difficulties which could reasonably be ascribed to an effect of dicoumarol on the transaminase values.

Summary and conclusion

Ten normal persons and 4 patients with stasis of the liver due to heart failure were examined for SGO-T and SGP-T four to five days after administration orally of 500 mg dicoumarol. In one very sick patient only with severe stasis of the liver a moderate increase to pathological values chiefly of SGO-T was observed.

The conclusion must be that administration of dicoumarol over a short period usually has no appreciable effect on the serum transaminases, and the assertion that a noxious effect on the liver by anticoagulant therapy may simulate a second myocardial infarction about three days after the first should be accepted with some reservation.

Acknowledgement

I am indebted to Rudolf Kelding, M.D., Head of the Central Laboratory, and to Mrs. Anne Holst for their kind assistance.

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Table I *Transaminase values in normal persons and in patients with stasis of the liver before and after administration of dicoumarol. Experiments 1—10 were made in normal persons, experiments 11—14 in patients with advanced stasis of the liver*

Case	Age	Sex	Test	Before administration of dicoumarol	Days after administration of dicoumarol 1	2	3	4	5
1	48	M	SGO-T	20	21	15	9	13	12
			SGP T	10	8	12	8	8	10
			PI	100	72	84	76	78	74
2	25	M	SGO-T	19	18	18	17	17	—
			SGP T	13	14	12	11	16	—
			PI	86	67	74	80	67	—
3	68	M	SGO-T	9	11	11	7	7	—
			SGP T	5	—	8	6	6	—
			PI	90	90	60	83	100	—
4	58	F	SGO-T	9	18	7	13	8	—
			SGP T	3	12	3	9	1	—
			PI	100	81	51	69	66	—
5	46	F	SGO-T	13	13	15	9	13	12
			SGP T	4	4	5	4	1	7
			PI	100	71	90	77	100	100
6	41	F	SGO-T	17	17	22	15	15	—
			SGP T	39	33	21	23	27	—
			PI	91	79	85	75	95	—
7	57	F	SGO-T	7	16	9	7	6	—
			SGP T	4	8	3	3	4	—
			PI	95	79	49	41	42	—
8	58	F	SGO-T	15	14	18	14	8	—
			SGP T	3	3	2	4	1	—
			PI	100	70	70	82	84	—
9	57	F	SGO-T	13	19	13	8	8	—
			SGP T	2	8	7	2	3	—
			PI	100	70	89	95	84	—
10	29	F	SGO-T	6	6	9	8	9	—
			SGP T	1	—	1	5	1	—
			PI	94	73	67	77	84	—
11	50	M	SGO-T	9	12	—	11	10	—
			SGP T	3	6	7	6	6	—
			PI	90	58	47	63	69	—
12	66	F	SGO-T	19	23	18	15	10	15
			SGP T	6	3	5	12	4	6
			PI	88	60	37	35	33	49
13	74	F	SGO-T	18	14	22	40	24	20
			SGP T	6	12	8	8	7	11
			PI	95	100	89	94	83	94
14	55	F	SGO-T	30	55	86	34	33	38
			SGP T	7	29	58	37	33	34
			PI	82	65	51	56	65	32

SGO-T = Serum-glutamic-oxaloacetic transaminase (normal value < 40 units).
SGP T = Serum glutamic-pyruvic transaminase (normal value < 35 units)
PI = Prothrombin index (normal range 80—120)

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Results are shown in table I. In patients 1—13 no or only a slight increase of either of the transaminase values was observed, no case rising to pathological values and, in particular no increase of SGP T Patient No 11 who suffered from very severe stasis of the liver is especially significant. Only patient No. 14 showed a pathological increase of both of the transaminase values. In this case the increase of the transaminase values coincided with a clinical aggravation of the condition which judging from symptoms and electrocardiographic findings presumably was due to pulmonary embolism or thrombosis.

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Polypoid Tumours of the Cardiac Auricles

Report of 3 Cases

By

Steen Olsen P Bach Nielsen and Jørgen Piper

Tumours of the heart are rare. The great majority of those on record have been published as case reports. It is difficult to gain an exact impression of the incidence. Searching the literature Pollia & Gogol, in 1936, found 154 *primary cardiac tumours* in 46,000 autopsies, an incidence of approximately 0.33 %. For various reasons, int. al. the mode of selection, this percentage must be considered too *high*, as stated also by Pollia & Gogol. Benjamin (1939) in 40,000 autopsies found 12 primary cardiac tumours, whereas Scott & Garvin, in the same year found no case among 11,000 autopsies and Straus & Merliss, in 1945 found no primary tumours of the heart in 36,000 autopsies. Although the exact incidence of primary cardiac tumours is unknown, the named studies have revealed that the condition is extremely rare *Secondary* (metastatic) *tumours* of the heart are considerably more common, but nevertheless the heart is one of the organs which appears to be least apt to house metastatic tumours. Considering the often very limited extent of the metastases the reported incidence of secondary cardiac tumours must depend to a marked extent upon the care with which the heart has been studied in the post-mortem series concerned. Prichard (1951) in 4,375 autopsies on patients with cancer found myocardial metastases in 3.4 %.

The diagnosis is seldom made during life. As a rule, a clinical diagnosis is merely of academic interest. In cases of benign growths, however there is a possibility of curative surgery. This applies particularly to polypoidal myxoma which is usually located in the atrium. Since Crafoord, in 1955 successfully removed a cardiac myxoma, an increased number of reports on such operations have appeared.

However tumours other than the myxomas may occur in polypoid form, simulating myxoma anatomically as well as clinically Malignant tumours of the

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Fig 1 Case 1 Right-sided carotid angiography showing a complete occlusion in the middle cerebral artery 3 cm from its origin.

heart (primary as well as secondary) usually affect the myocardium, but (as in 2 of our cases) they may form polypoidal prominences in the cavities of the heart

The following 3 cases of polypoid tumours of the auricles showed certain striking clinical and pathological features.

Case reports

Case 1 A married female clerk, aged 36 was admitted to Bispebjerg Hospital, Dept C on July 20 1960. On the day before admission she had suddenly lost consciousness and was brought to the neurosurgical department Carotid arteriography revealed total occlusion of the right middle cerebral artery 3 cm from its origin (cf. fig 1)

It was reported that once or twice a month during the past year she had suffered from syncopal attacks. There had also been a few episodes of sudden aphasia of a few minutes duration. During the same period her appetite had been poor she had lost 17 kg and had a tendency to palpitations. There was no history of rheumatic fever or severe infectious diseases, and she had not had exertional dyspnoea. No pregnancies.

Physical examination now revealed left sided central facial palsy and moderately severe left-sided hemiparesis and hemihyperaesthesia. Auscultation of the heart disclosed a thrill over the apex, a presystolic murmur a split and accentuated P_2. The heart rhythm was regular She received physiotherapeutic treatment and anticoagulant medication, initially with good results. On Aug 12th she had another cerebral episode with exacerbation of the left-sided paresis, and on Sept. 1/ she had tonic-clonic convulsions of 31/2 hours duration. Two days later she died. Laboratory findings Hb. level 82—73 %, white blood cells 10,500—11 700, red and white blood picture normal, E. S. R. 16—96 mm/hour antistreptolysin titre and antistreptococcal hyaluronidase titre normal, W. R. negative. Spinal fluid showed normal cell count and protein and the urine neither albumin nor sugar Temperature normal or slightly elevated, pulse rate between 70 and 90, blood pressure 95/70—110/80 mm Hg Chest radiography showed the size and shape of the heart to be within the normal range The heart measured 11 0 cm, the chest 22.5 cm. The lung fields were normal. ECG (cf. fig 2) on July 23rd Rate 88, P—Q interval 0 12 sec. frequent supraventricular extrasystoles with negative, pointed P waves in the second limb lead.

ECG (Aug 27th) Rate 75, P—Q interval 0 10 sec. T isoelectric in the second limb lead and in V_4, T negative in V_4 and V_5

The condition was interpreted as mitral stenosis with cerebral embolic episodes. Her transfer to a department of thoracic surgery was being prepared when she died.

Post-mortem findings

The heart measured 10 × 10 cm. The pericardium was normal, without coatings or accumulations of fluid After being opened, the left atrium showed a polypoid tumour of a soft, gelatinous consistency and of a light orange colour It measured approx. 1×1×3 cm and arose from the septum (cf. fig 3) This pedunculated tumour projected down into the mitral orifice Other parts of the endocardium, in the left atrium as well as in all other chambers were normal, and the valves

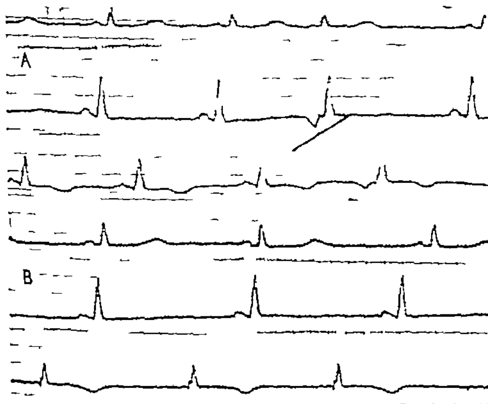


Fig. 2. Case 1. A. ECG on 23. 7. 1960. B. ECG on 27. 8. 1960 (limb leads)

were entirely normal. In particular there was no sign of mitral valvular abnormality. The myocardium was not thickened. In the posterior wall of the left ventricle there was a rather ill-defined fibrous area, 2 × 3 cm. The coronary arteries proved normal, without atheromatous or stenoses. No embolus or thrombus could be found in the arterial branch leading to the infarction scar.

Other organs were characterized by moderate chronic cardiac congestion. The lungs were oedematous. Infarcts were found in *both kidneys*, and in the *spleen* there was a fresh ischaemic infarction.

On *microscopic examination* the tumour of the left atrium was a typical myxoma. Its surface was lined with flat, endothelial cells. The stroma, which was rather acellular, consisted of homogeneous, slightly PAS positive basic substance with a moderate number of capillaries and a few fibroblast-like cells. At the base there was a moderate content of collagen fibrils. Otherwise, the basic substance was practically homogeneous. Alcian staining revealed a moderate but unmistakable positive reaction for mucous.

Brain. In the main trunks of both middle cerebral arteries were ample quantities of soft, gelatinous masses resembling emboli, which were grossly of the same nature as the cardiac tumour. These masses were fairly easy to push out of the vessels. No abnormalities of the brain stem or cerebellum. The surface of the cerebrum showed a soft, retracted area infero-posteriorly in the right frontal lobe and anteriorly in the right parietal lobe corresponding quite closely to the area supplied by the middle cerebral artery. In the symmetric part on the left there was a slight dark discolouration and softening. Frontal section revealed, at the site of the surface change on the right, breakdown of the tissue which looked like fresh softening. The necrotic area extended fat the lenticular nucleus, destroy-



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Fig 1 Case 1 Right-sided carotid angiography showing a complet occlusion in the middle cerebral artery 3 cm from its origin.

heart (primary as well as secondary) usually affect the myocardium but (as in 2 of our cases) they may form poly poidal prominences in the cavities of the heart

The following 3 cases of polypoid tumours of the auricles showed certain striking clinical and pathological features

Case reports

Case 1 A married female clerk, aged 36 was admitted to Bispebjerg Hospital, Dept. C on July 20 1960 On the day before admission she had suddenly lost consciousness and was brought to the neurosurgical department Carotid arteriography revealed total occlusion of the right middle cerebral artery 3 cm from its origin (cf. fig 1)

It was reported that once or twice a month during the past year she had suffered from syncopal attacks. There had also been a few episodes of sudden aphasia of a few minutes duration. During the same period her appetite had been poor she had lost 17 kg, and had a tendency to palpitations. There was no history of rheumatic fever or severe infectious diseases, and she had not had exertional dyspnoea. No pregnancies.

Physical examination now revealed left sided central facial palsy and moderately severe left-sided hemiparesis and hemihyperaesthesia. Auscultation of the heart disclosed a thrill over the apex, a presystolic murmur a split and accentuated P_2. The heart rhythm was regular She received physiotherapeutic treatment and anticoagulant medication, initially with good results. On Aug 12th she had another cerebral episode with exacerbation of the left-sided pareses, and on Sept. 17 she had tonic-clonic convulsions of 3 1/2 hours duration. Two days later she died. Laboratory findings Hb. level 82—73 %, white blood cells 10,500—11 700, red and white blood picture normal, E. S. R. 16—96 mm/hour antistreptolysin titre and anti-streptococcal hyaluronidase titre normal, W R. negative. Spinal fluid showed normal cell count and protein and the urine neither albumin nor sugar Temperature normal or slightly elevated, pulse rate between 70 and 90 blood pressure 95/70—110/80 mm Hg. Chest radiography showed the size and shape of the heart to be within the normal range. The heart measured 11 0 cm, the chest 22.5 cm. The lung fields were normal. ECG (cf. fig 2) on July 23rd Rate 88, P—Q interval 0.12 sec., frequent supraventricular extrasystoles with negative, pointed P waves in the second limb lead.

ECG (Aug 27th) Rate 75, P—Q interval 0 10 sec., T isoelectric in the second limb lead and in V T negative in V and V_6

The condition was interpreted as mitral stenosis with cerebral embolic episodes. Her transfer to a department of thoracic surgery was being prepared when she died

Post mortem findings

The heart measured 10 × 10 cm The pericardium was normal, without coatings or accumulations of fluid. After being opened, the left atrium showed a polypoid tumour of a soft, gelatinous consistency and of a light orange colour It measured approx. 1×1×3 cm and arose from the septum (cf fig 3) This pedunculated tumour projected down into the mitral orifice. Other parts of the endocardium, in the left atrium as well as in all other chambers were normal, and the valves

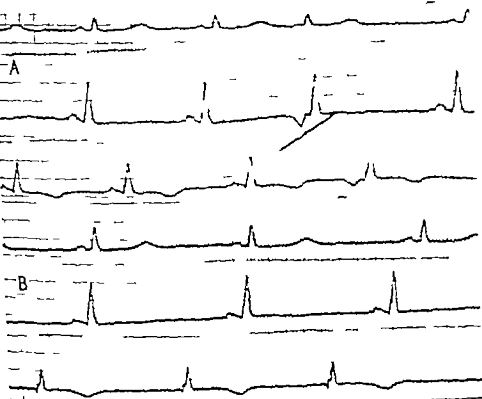


Fig 2. Case I. A. ECG on 23.7.1960. B. ECG on 27.8.1960 (limb leads)

were entirely normal. In particular there was no sign of mitral valvular abnormality. The myocardium was not thickened. In the posterior wall of the left ventricle there was a rather ill-defined fibrous area, 2 × 3 cm. The coronary arteries proved normal, without atheromatosis or stenoses. No embolus or thrombus could be found in the arterial branch leading to the infarction scar.

Other organs were characterized by moderate, chronic cardiac congestion. The lungs were oedematous. Infarcts were found in *both kidneys*, and in the *spleen* there was a fresh ischaemic infarction.

On microscopic examination the tumour of the left atrium was a typical myxoma. Its surface was lined with flat, endothelial cells. The stroma, which was rather acellular, consisted of a homogeneous, slightly PAS positive basic substance with a moderate number of capillaries and a few fibroblast-like cells. At the base there was a moderate content of collagen fibrils. Otherwise, the basic substance was practically homogeneous. Alcian staining revealed a moderate but unmistakable, positive reaction for mucous.

Brain. In the main trunks of both middle cerebral arteries were ample quantities of soft, gelatinous masses resembling emboli, which were grossly of the same nature as the cardiac tumour. These masses were fairly easy to push out of the vessels. No abnormalities of the brain stem or cerebellum. The surface of the cerebrum showed a soft, retracted area infero-posteriorly in the right frontal lobe and anteriorly in the right parietal lobe, corresponding quite closely to the area supplied by the middle cerebral artery. In the symmetric site on the left there was a slight dark discolouration and softening. Frontal section revealed, at the site of the surface change on the right, breakdown of the tissue which looked like fresh softening. The necrotic area extended into the lenticular nucleus, destroy-



Fig 3 Case 1 The heart opened on the left. In the upper right-hand corner the pedunculated auricular myxoma.



Fig 4 Case 1 The right middle cerebral artery with constituents of myxoma.

ing the insular cortex. In a separate site laterally in the occipital lobe there was a somewhat older necrotic lesion, approx. 3 cm in diameter of a yellowish-brown colour with multicystic cavities in the centre. In the left hemisphere there were two walnut-sized granular necroses, evidently of the same age as the large necrosis in the area supplied by the middle cerebral artery on the right. Microscopical examination showed the lumina of both middle cerebral arteries to be totally occluded by rolled-up myxomatous masses of exactly the same appearance as the cardiac tumour (cf. fig 4) Only in one site did these masses appear to be adherent to the vascular endothelium. The foci of softening in the brain were found to be of somewhat different age.

Case summary A 56-year-old woman, with a history of fainting spells and aphasic episodes, was admitted with left sided paresis and facial palsy Carotid arteriography revealed total occlusion of the right middle cerebral artery Cardiac auscultation indicated mitral stenosis, but the X ray appearances of the heart were normal. ECG revealed frequent supraventricular extrasystoles, a P—Q interval of 0 10 sec., and a negative T in V_1 and V_2 The disease was interpreted as mitral stenosis with cerebral emboli. After another cerebral episode the patient died.

Autopsy revealed a pedunculated, gelatinous tumour in the left atrium, projecting down into the mitral orifice.

Microscopical examination Myxoma. In the brain, in both middle cerebral arteries gelatinous, embolus-like masses of the same microscopic appearance as the cardiac tumour Moreover several foci of softening

Case 2. A 50-year-old semi-skilled worker was admitted on Oct. 28th, 1960 to the medical department of Ringsted Hospital from the County Hospital in Haslev

No history of rheumatic fever or venereal diseases. During the past month he had been suffering from a feeling of oppression in the upper part of the epigastrium and behind the sternum, but had not had palpitations, exertional dyspnoea, cough, edema or petechiae There had been one episode of short-lasting malaise associated with giddiness. For the past fortnight he had been running a temperature of up to 38 C.

On admission he was looking well without cyanosis, dyspnoea or anaemia. Cardiac aus-

cultation showed normal limits, with the apex impulse within the mid-clavicular line. No thrill or pressure. At the apex a long, moderately strong, rolling diastolic murmur ending in an accentuated first sound. At the apex and inferiorly at the left sternal border the second heart sound was split. The P was accentuated, heart rhythm regular 80. Other objective findings normal. A few days after admission he began to have dry cough, later productive of ample bloody sputum. His general condition rapidly deteriorated, he developed increasing dyspnoea and cyanosis, the heart rate gradually rose to 130, and the blood pressure fell. On Nov 10 the patient died in state of *pulmonary edema*, 6 weeks after the onset of the illness.

Laboratory findings: Hb. level 83 % white blood cells 10,000 with normal differential count. E. S. R. 65 mm, antistreptolysin titre and antistreptococcal hyaluronidase titre normal, cultures of venous blood sterile. Urine without albumin, blood, or sugar Sterile pyuria, no tubercle bacilli on culture. Blood pressure between 110/55 and 90/35 mm Hg, temperature constantly around 38° C.

Chest radiography showed the heart to be of normal size and configuration, measuring 12 cm (chest 31 cm), lung fields normal. On Nov 7th the X-ray films showed severe, diffuse blurring of both lung fields, most marked towards the hila, and small effusion in the right pleural cavity The cardiac size was unchanged, 12 cm against the chest width 31 cm.

ECG (Oct. 29th) Rate 80, regular sinus rhythm, P—Q interval normal (0.16 sec.)

ECG (Nov 10th) Rate 130, but otherwise normal findings.

The patient was treated with penicillin, diuretics, coddanid, and pethidine. The condition was interpreted as mitral stenosis with cardiac failure, possibly caused by subacute bacterial endocarditis.

Post-mortem findings

The heart measured 11 × 10 × 6 cm, weight 375 g. After it had been cut open an egg-shaped intumescence with smooth surface, measuring 7 × 4 × 4 cm, was found in the left atrium (fig. 5) The tumour was attached to the septal wall of the atrium by fairly thick stalk and projected into the mitral orifice which, however was not stenosed. The myocardium was 5 mm in thickness on the right and 10—12 mm on the left side where a pea-sized whitish infiltration was found. The coronary arteries were entirely normal. Furthermore, the autopsy showed mild chronic congestion of the organs and severe pulmonary edema. In the right *adrenal* there was a cherry-sized whitish-yellow tumour of soft, almost liquid consistency In the *left kidney* there was pea-sized whitish infiltration.



Fig 5. Case 2. A large polypoid tumour issuing from the septum in the left atrium.



Fig 6. Case 2. Histological structure of the atrial tumour (metastasis from phaeochromocytoma).



Fig 7 Case 3 ECG limb leads from 21 10 1960.

Microscopical examination (fig 6) revealed that the tumours in the adrenal and in the atrium and myocardium were of the same architecture, made up of oval or polygonal epithelial cells with varying quantities of granular cytoplasm. The nuclei were large, of a high chromatin content, slightly poly morphous with distinct nucleoli. Numerous mitoses. The adrenal tumour evidently origi nated in the medulla. In the periphery there was compressed cortical tissue.

Microscopical diagnosis Malignant phaeochromocytoma of the right adrenal with polypoid metastases in the left atrium (ad. Soeborg Ohlsen)

Case summary A 30-year-old man was admitted with persistent fever and auscultatory signs of mitral stenosis, while the ECG and radiographic appearances of the heart were normal. He died in a state of pulmonary edema.

Autopsy showed the left atrium to be filled with a pedunculated tumour $7 \times 4 \times 4$ cm, projecting down into the mitral orifice. In the right adrenal gland there was a whitish yellow tumour the size of a cherry

Microscopical diagnosis Malignant pheochromocytoma of the adrenal with a poly poid metastasis to the left atrium

Case 3. A 66-year-old basket maker was ad mitted to Bispebjerg Hospital, Med. Dept. C with duodenal ulcer on June 23rd, 1960

In 1951 he had been admitted to the same department with cardiac neurosis and cervical spondylosis. At that time cardiac auscultation and ECG were normal, the W R. negative and the blood pressure 135/80. Since the age of 25 the patient had been suffering periodically from dyspepsia, during the past months vomiting and for some weeks also epigastric pain. For 3 or 4 months there had been pain in the left chest and in the left arm. A fortnight before admission he had noted enlargement of the lymph nodes in the left axilla and left groin. No weight loss and no cardio-pulmonary symptoms.

His general health and nutritional state were good. Cardiac auscultation normal. On the neck, in the axillae and in the left groin, a few lymph nodes were palpable. They were firm some of them hard, and up to plum size. No hepato- or splenomegaly Other objective findings normal

Laboratory findings Hb. 100 — 91 %, white blood cells 5,900 with a normal differential count. Weight 61 8 kg, height 173 cm. Blood pressure 135/65 urine without albumin or sugar

Histological examination of a lymph node from the left axilla revealed lymphosarcoma.

X rays of the stomach disclosed an ulcer crater as large as a pea, in the pyloric area. Histamine test showed free acid in the stomach Benzidine test of the faeces gave alternating positive and negative reactions.

Chest radiography showed the cardiac shadow to be of normal shape and size measuring 13.5 cm compared with the chest 27.5 cm. Mediastinal shadow 6 cm.

ECG normal, rate 62 (fig 7 three top curves)

The patient was treated by a dietary regimen and X ray therapy to the axillae and groins. Discharged on July 29 1960

In the middle of August 1960 he began to have increasing dyspnoea, cough, dysphagia, and on a few occasions vomiting He was readmitted on Sept 14 On this occasion, his general condition had deteriorated, his skin had grown paler and he was running a low grade fever Cardiac auscultation normal. Chest radiography now showed a marked swelling of the mediastinum, mainly in front of the trachea. Th width of the mediastinum was 8.5 cm, the cardiac shadow diffusely enlarged to 15 5 cm, chest 27.5 cm, lung markings increased, presumably because of congestion The right costo-phrenic angle was obliterated

ECG (21st Oct.) Rate 42, nodal rhythm. P—Q interval 0.13 sec., P_1 notched, P and P negative.

Other findings Hb. 58 %, white blood cells 4 000 with normal differential count, E. S. R. 78, 101, 79 mm/hour weight 61 kg. Blood pressure 115/60 mm Hg, temperature slightly elevated, pulse rate about 50—60.

He was treated with X-ray irradiation to the neck and mediastinum. His general condition went steadily downhill, and he died on Oct. 26th 1960 without the nature of the heart disease having been clarified.

Post-mortem findings

In the chest were greatly enlarged, adherent lymph nodes with tumour like changes in both hili. These nodes ranged in size from hazel nut to green walnut they were fairly soft, uniform and whitish on cut section. The *oesophagus* was compressed, but did not show invasion. In the *abdominal cavity* too there were large, adherent, soft lymph nodes, chiefly in the porta hepatis and along the lesser curvature of the stomach. When the *heart* was opened, the entire right atrium was found to be filled with large neoplastic masses forming rounded nodules ranging in size from pea to walnut (fig. 8) The tumour was adherent and attached by a fairly thin pedicle to the endocardium in the area of the septum. The cardiac valves and orifices as well as the remaining parts of the endocardium were normal. So was the myocardium. In particular there were no neoplastic infiltrations in the myocardium.

Microscopical examination. The architecture of the enlarged lymph nodes and the polypoid neoplastic masses in the right atrium was identical. The tumour tissue was extremely cellular small-celled, of mesodermal nature. Wilder staining showed only a very few reticular fibres.

The *diagnosis* was, therefore, thoracic and abdominal lymphosarcoma with polypoidal growth of lymphosarcoma from the wall of the right atrium.

Case summary A 66-year-old man was admitted with enlargement of the lymph nodes on the neck, in the axillae, and in the left groin. Microscopical



Fig. 8. Case 3. Right atrium packed with soft polypoidal masses.

tion revealed lymphosarcoma. Within a few months the heart increased in width and a large swelling developed in the mediastinum. ECG showed an atrioventricular conduction disturbance with bradycardia, presumably nodal rhythm. Autopsy revealed a number of lymphosarcomatous lymph nodes in the mediastinum and abdominal cavity The right atrium was packed with a pedunculated tumour mass whose microscopical appearance was identical with that of the lymph nodes.

Discussion

In case 1 a so-called myxoma was found in the left atrium. We do not propose to enter into the heated discussion regarding the true nature of these polypoid masses, whether they are thromboses organized in a way differing from the usual manner or whether they are actual connective tissue tumours. In recent years, the latter view has prevailed.

Our case illustrates the differential

polyp may be expected to present owing to the pathological changes caused by it. The prominence of the polyp into the mitral orifice compromises the flow through this orifice and gives rise to symptoms which are bound to lead to a clinical diagnosis of mitral stenosis. In addition to the cardiac symptoms and signs, there is the occurrence of emboli to be considered in our case in the cerebral arteries. In the presence of mitral stenosis due to endocarditis such emboli may be explained by the detachment of parietal thrombi from the dilated atrium or by endocarditic activity (subacute endocarditis in a healed rheumatic lesion) thrombotic masses being detached from the valves. It is quite characteristic that our patient was first admitted to a neurosurgical department because of the acute cerebral episode. The syncopes preceding the severe attack which led to admission may be explained by acute circulatory disturbance due to myxomatous masses in the orifice or due to small cerebral emboli Autopsy showed, moreover signs of previous emboli in the kidneys, spleen, and myocardium, changes which could not be correlated with the clinical course. Embolic episodes in the presence of atrial myxoma may originate in two ways

1 Detachment of thrombi from the surface of the tumour or possibly from the walls of the atrium where the circulation must be assumed to be abnormal, or

2 Detachment of bits of the soft, frequently laciniate tumour

In our case the latter mechanism applied as is evident from the demonstration of tumour tissue upon microscopical examination of cerebral arteries.

Our second case exemplifies how a metastasis in the heart may assume the form of a polyp and thus give rise to a symptom complex reminiscent of case 1 The patient was a youngish man who rapidly developed signs of cardiac failure. Auscultation indicated mitral stenosis, while the rapid progression the fever the elevated E. S R., and the leukocytosis suggested subacute endocarditis. Blood cultures were negative. This patient did not have embolic symptoms. Death occurred after heart failure with pulmonary oedema. Autopsy showed, as in case 1 a polyp attached to the wall of the left atrium. So far the two cases were almost identical, except for the absence of emboli in case 2 Microscopical examination however showed unexpectedly malignant tumour tissue of epithelial nature. The histological structure as well as the presence of a tumour of the same architecture in one adrenal gland appear to justify the diagnosis of pheochromocytoma. As the tumour had been fixed in formalin, a chromaffin reaction could not be performed. Hypertension had not been found clinically and indeed this could hardly be expected in view of the mitral occlusion. The diagnosis of pheochromocytoma is based exclusively on the characteristic histological appearance and the presence of an adrenal tumour The same applies to a number of reported cases of pheochromocytoma (Sherwin 1959)

Our third case was a polypoid *lymphosarcoma* issuing from the wall of the right atrium and projecting into the tricuspid orifice. In addition to the cardiac tumour the patient had enlarged lymph nodes in the chest and in the abdomen. Similar cases have been reported by Somers & Lothe (1960) who described 3 cases of primary lymphosarcoma of the *heart*, two of them polypoid projecting *into* the tricuspid orifice. Other cardiac sarcomata may assume this form (cf Ma

haum 1945 and Somers & Lothe 1960) among others the closely related reticulosarcoma (Komczynski 1957) In this group of tumours, there does not seem to be much point in distinguishing between primary and secondary tumour growth. In Somers & Lothe's case the cardiac tumour was larger than the others. In our case there were, apart from the atrial tumour widespread lymphosarcomatous masses hailing from thoracic and abdominal lymph nodes. Regardless of the mutual size relation of the different tumour areas, it seems likely that the growth had originated in the lymphoid tissue and metastasized to the heart, although the cardiac tumour did assume greater dimensions than those in the neighbouring lymph nodes. At least, the literature does not contain any reports on lymphosarcoma localized exclusively to the heart.

Up to the present time, most cases of cardiac tumours have in fact been merely of theoretical interest to the clinician. Now the increased possibilities of surgery have afforded a chance of curing some of these cases, particularly the benign polypoid myxomata. The diagnosis is still very difficult. A knowledge of the pathology and clinical features of the condition, however gives some chance of making this diagnosis during life (Kirkeby & Leren 1952) In particular the diagnosis must be borne in mind when youngish persons without a history of rheumatic fever rapidly develop heart failure with auscultatory signs of mitral stenosis, possibly episodes of emboli or weakness, in some cases may be Adams-Stokes attacks due to cerebral hypoxia caused by occlusion of the mitral orifice by the tumour when the patient bends forward. Hypotension is a common finding The diagnosis may be further supported by certain auscultatory subtleties, int. al. the fact that the murmurs alter with changes in body position. Most cases, however will still be diagnosed either by *angiography* or in the course of operation, the surgeon expecting mitral stenosis, but finding instead a polypoid tumour

There are no *ECG findings* characteristic of atrial tumours. Some patients have right axis deviation (Harvey 1957) others supraventricular conduction disturbances and extrasystoles like two of our cases. In others again, the electrocardiogram may be normal.

Our *cases 2* and *3* illustrate how malignant tumours of the heart may assume polypoidal form and thus imitate myxoma, pathologically as well as clinically In such instances, surgery is hardly indicated, unless all the findings suggest that metastases have not spread to other sites.

Summary

Three cases of polypoid tumours of the cardiac auricles are described

1 A 36-year-old woman with a myxoma of the left auricle and tumour emboli int. al. to the brain, demonstrated by angiography as well as post mortem.

2. A 30-year-old man with polypoid metastases from a pheochromocytoma to the left auricle.

3 A 66-year-old man with a polypoid growth of lymphosarcoma in the right auricle.

The clinical and post-mortem findings are described, and the possibilities of diagnosing the tumours during life are discussed

Polypoid tumours of the cardiac auricles are in principle operable, but benign

myxomata are not the only tumours which may assume this form as secondary tumours may also appear as polypoid growths in the heart.

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We are also indebted to Prosector A. Soeborg Ohlsen, M D for the patho-anatomical description of case 2

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The Pulmonary Rate of Uptake of Radioactive Argon and Ethyliodide

A Study on Healthy Subjects and on Patients with Lung Disease

By

H. Colldahl, T. Alvåger, J. Uhler and G. H. Neumann

In earlier investigations the simultaneous elimination rate of gases with different physical constants has been investigated. Thus Colldahl, Alvåger and Uhler showed in 1959 and 1960 that acetylene, argon and xenon are eliminated at about the same speeds which, however are lower for patients with certain stages of heart and lung diseases compared with healthy subjects. A small, but nevertheless significant delay in the elimination of argon or xenon compared with acetylene could be shown in certain patients with sarcoidosis or silicosis, in which one may expect to find lung fibrosis. This discrepancy may be a sign of impaired diffusion in these patients. In patients with pronounced emphysema the elimination rates were low but both the injected gases were eliminated at about the same rate

Liljestrand and Sahlstedt in 1925 carried out an experimental investigation into gas diffusion through frog lungs which were enclosed in a gas chamber and exposed to different gas mixtures either inwards through the air tubes or outwards from the surrounding gas chamber The following relative diffusion values were obtained

CO	N	82.7
CO	O	39 2
C_2H	O	46.0
C_2H_2	CO	1 36

As a comparison with these values Liljestrand and Sahlstedt also made use of the relative values of diffusion, calculated from the absorption coefficient of the gas in water divided by the square root of the molecular weight. The relative values of the diffusion of the nitrogen, oxygen, carbon dioxide and acetylene are then 1 1.9 35 88 at a temperature of 15

Looking at our previous results, together with those of Liljestrand and Sahlstedt, we might say that diffusion does not seem to be a limiting factor

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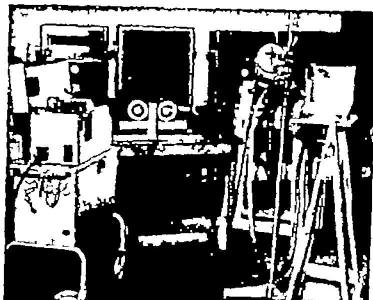


Fig. 1. The apparatus used.

On the left is the recording mingograph, in the middle the two γ-scintillator detectors. In the background are seen the pulse height analysers and scales. On the right the breathing valve is seen together with gas meters and a lead protection for the bag in which the air breathed out is collected.

as regards the elimination of gases from the blood to the lungs. Instead circulation and ventilation may be said to be of decisive importance in the living organism.

In order to have a clearer understanding of the laws governing gas uptake and elimination through the lungs it was also felt worth-while to study simultaneously the pulmonary rate of uptake of gases with different physical constants. If one wishes to study the uptake during the first 30—60 seconds after the inhalation of the gases the best way perhaps is to use two radioactive gases with different kinds of radiation so that both gases can be independently detected simultaneously in one breathing test. In our experiments we have used radioactive argon (Ar^{41}) and radioactive ethyliodide (I^{131}). The water solubility of ethyliodide (0.4 g/100 ml H_2O 20° C) is about the same as that of acetylene and carbon dioxide (4) and the solubility of argon is nearly the same as that of oxygen (4). Ethyliodide has previously been used in the study of lung function (4). Radioactive ethyliodide was used for lung function studies in 1953 (6) and radioactive methyliodide has been employed in so-called inhalation radiocardiography (5).

Method

The same equipment was used as in previous experiments (2) apart from the employment of two measuring units, each of them consisting of a γ-scintillation detector, a pulse height analyser and a scale, to enable simultaneous and independent readings of two gases to be taken. In the actual experiment one of the detector units discriminates all but high energy γ-rays (about 1 MeV) to detect Ar^{41} and the other unit measures only γ-radiation of about the energy 360 KeV that is typical for I^{131}.

Argon in small quartz bulbs was irradiated in a reactor and the activated gas (Ar^{41}) was then diluted with air in rubber bags. Radioactive ethyliodide (I^{131}) in glass ampoules from The Radiochemical Centre, Amersham, England, was used and was introduced into plastic bags by squeezing the ampoules in a rubber tube connecting the bags so that the air was transferred from one bag to the other.

In the respiration tests the patients hap to inhale one or a mixture of two radioactive gases from a plastic bag. First the patient exhaled as completely as possible after which the bag was coupled to the inhaling side and then the patient breathed in as deeply as he

Table I

Patients	Born	Diagnosis	Vital capacity		Date of examination
H.E.H.	17 05 20	Polyarthritis levis	4.1	4.2	23/6 1960
L.G.O.	21 12 19	Tbc pulm. sin.	2.7	2.7	23/6 1960
P.T.	31 11 05	Emphysema pulm. + cardiosclerosis + insuff. cordis	1.5	1.3	10/11 1960
E.A.	04 04 22	Asthma bronch. + emphysema pulm. + sinusitis max. bilat.	3.5	—	17/6 1960
K.K.G.	03 10 09	Bronchopneumonia + asthma bronch. + emphysema pulm.	1.3	1.2	17/6 1960
K.O.	13 05 28	Asthma bronch. + status post. tbc pulm.	—	—	7/10 1960

as able and held his breath as long as he could. By attaching the patient exhaling tube to gas meter it was possible to ensure that no exhalation occurred while he was holding his breath.

The two γ-scintillators are shielded from each other and from their surroundings by means of lead tubes and are as a rule placed on the patient back with the centre directly under the angle of the shoulder blade and equidistant from his spinal column, on each side. (The apparatus is shown in fig 1.)

The concentration of the gases is recorded on kymograph of the mingograph type and shows the counting rates of the detectors with time constant of 0.1—1 sec.

Some 50 tests have been carried out on altogether 35 healthy subjects and patients with different lung diseases, especially asthma. The patient has inhaled between and 20 μC of the radioactive gases. The elimination of ethyliodide in the urine has occurred mainly during the following 24 hours and amounts of —10 μC have been observed.

The mean values of the elimination rates expressed as percentage per second for the different gases has been determined for the time interval 6—14 sec. after the tracer gases have reached the lungs (fig the curves begin to rise). For the determination of the percentages the curve height point 10 seconds after the tracer gases has reached the lungs has been used (table II).

In the curves here presented inhalation of radioactive gas has been marked thus ↓ and exhalation thus ↑. The patient has held his breath between the arrows. Each set of curves contains one or more calibration tests where the two measuring units are compared with each other as regards the height of the curve for the same amount of radioactivity. Peaks on the curves without arrows show such calibration tests.

The curves have always the same rate (1 cm = 2 sec.) and have for technical photographic reasons been divided into 2 or more lengths which always follow directly after each other. The curve lines for the right lung is the lower one, that for the left lung the upper one in the tests when one detector was placed on each lung. In order to protect the thyroid the patient was given 0.5 g of potassium iodide before inhaling the radioactive ethyliodide.

Results

I THE INHALATION BY HEALTHY PERSONS OF ONE GAS AT A TIME. ONE DETECTOR ON EACH LUNG

The following patient is given as an example

H. E. H. on 23/6 and 27/6 1960 (table I)

a) Radioactive ethyliodide (I^{131}) was measured on both sides. The curve lines run parallel and follow each other. After reaching the maximum the curves begin to sink relatively quickly. Even after the patient has continued breathing for a relatively long period the curve does not reach the base line since some of the radioactive ethyliodide is retained in the body (fig)

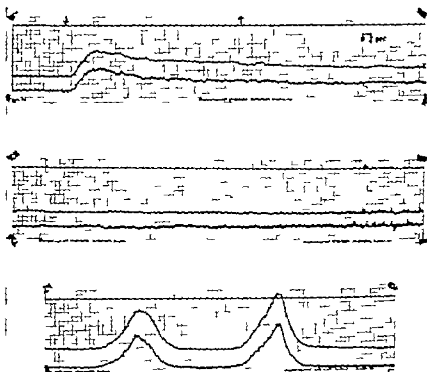


Fig. 2. Pat. H. E. H. (Polyarthritis levis)

Curve 1 Radioactive ethyliodide was inhaled and measured on both sides. The upper curve is from the left lung and the lower from th right. The patient held his breath between the arrows; Curve 2: Continuation of curve 1 Curve 3 Callibration tests. Both detectors registered ethyliodide.

b) Argon measured in both lungs. The maximum is reached after a relatively short time. Then the curve line sinks slowly until breathing starts, whereupon the curve rapidly reaches the base line (fig 3)

II Inhalation of one gas at a time by patients whose breathing is very different in both lungs

One detector on each lung

As an example we have chosen patient L. G O whose left lung is only partly efficient (table I)

a) Inhalation of ethyliodide on 23/6 1960

Activity reaches a normal peak in the right lung Thereafter the reduction is more pronounced than normal. In the left lung only a small increase is observed and the maximum is reached slowly (fig 4)

b) Inhalation of radioactive argon (27/6 1960) gives a normal peak for the right lung The peak for the left lung is insignificant (fig 5)

III Inhalation of radioactive ethyl iodide by patients with pronounced lung changes

One detector on each lung

The following patient is taken as an example P T on 10 11 1960 (table I)

This curve differs from that of a normal person in that the drop after the maximum is reached is less pronounced in the first stage than in the later stage (fig 6)

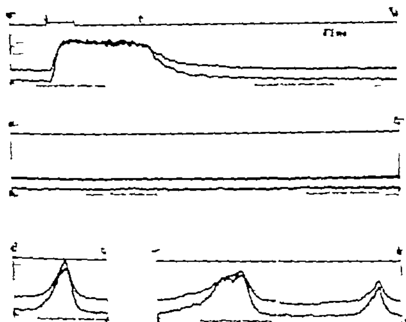


Fig. 3. Pat. H. E. H. (Polyarthritis levis)

Curve 1. Radioactive argon was inhaled and measured on both sides. The upper curve is from the left lung and the lower from the right. Curve 2. Continuation of curve 1. Curve 3. Calibration tests. From these one can see that in this experiment the amplitude of the lower curve is larger than that of the upper one for the same radioactivity. Both detectors registered argon.

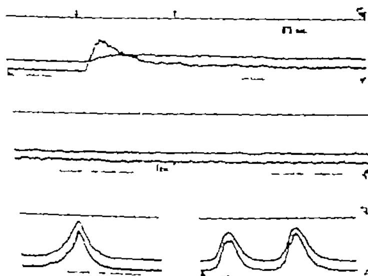


Fig. 4. Pat. L. G. O. (Tbc. pulm. sin.)

Curve 1. Radioactive ethyliodide was inhaled and measured on both sides. The upper curve is from the left lung and the lower from the right; Curve 2. Continuation of curve 1. Curve 3. Calibration tests. Both detectors registered ethyliodide.

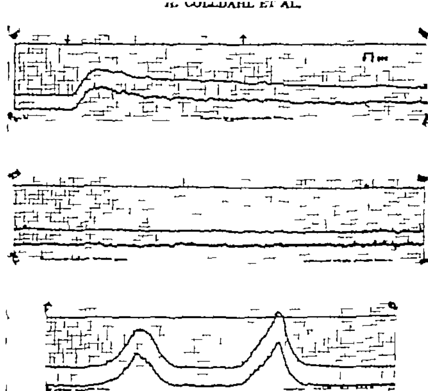


Fig 2. Pat. H. E. H. (Polyarthritis levis)

Curve 1: Radioactive ethyliodide was inhaled and measured on both sides. The upper curve is from the left lung and the lower from the right. The patient held his breath between the arrows. Curve 2: Continuation of curve 1; Curve 3 Calibration tests. Both detectors registered ethyliodide.

b) Argon measured in both lungs The maximum is reached after a relatively short time. Then the curve line sinks slowly until breathing starts, whereupon the curve rapidly reaches the base line (fig 3)

II Inhalation of one gas at a time by patients whose breathing is very different in both lungs

One detector on each lung

As an example we have chosen patient L. G O whose left lung is only partly efficient (table I)

a) Inhalation of ethyliodide on 23/6 1960

Activity reaches a normal peak in the right lung Thereafter the reduction is more pronounced than normal. In the left lung only a small increase is observed and the maximum is reached slowly (fig 4)

b) Inhalation of radioactive argon (27/6 1960) gives a normal peak for the right lung The peak for the left lung is insignificant (fig 5)

III Inhalation of radioactive ethyl iodide by patients with pronounced lung changes

One detector on each lung

The following patient is taken as an example P T on 10/11 1960 (table I)

This curve differs from that of a normal person in that the drop after the maximum is reached is less pronounced in the first stage than in the later stage (fig 6)

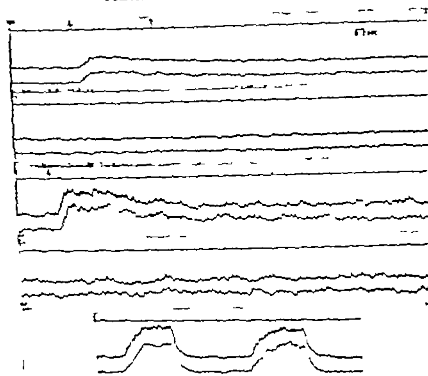


Fig. 6. Pat. P. T. (Emphysema pulm.)

Curve 1. Radioactive ethyliodide was inhaled and measured on both sides. The upper curve is from the left lung and the lower from the right. Curve 2. Continuation of curve 1. Curve 3. Another inhalation of radioactive ethyliodide was measured and registered with greater magnification than in curve 1. Curve 4. Continuation of curve 3, Curve 5. Calibration tests. Both detectors registered ethyliodide.

V. INHALATION OF TWO GASES SIMULTANEOUSLY IN PATIENTS WITH PRONOUNCED LUNG CHANGES

Both detectors on one side.

As an example can be mentioned the curve for a patient K. K. G. (17/6 1960). Here it will be seen that the curve line for argon follows that for the previous patient, while the slope of the curve for ethyliodide is less pronounced in its early portion (fig. 8).

Table III

Patients	Date of examination	Diagnosis	Argon eliminated in 60 sec. (%)	Acetylene eliminated in 60 sec. (%)
H. G.	20/3 1959	Anaemia	56	59
A. M.	6 3 1959	Sclerosis	40	50
B. N.	6 3 1959	Pneumonia	44	42
O. C.	12 5 1959	Emphysema	23	26

Discussion

In this investigation the uptake through the lungs during the first seconds after inhalation has been studied simultaneously for different gases with very different solubilities in water. The uptake has been studied when the patient has

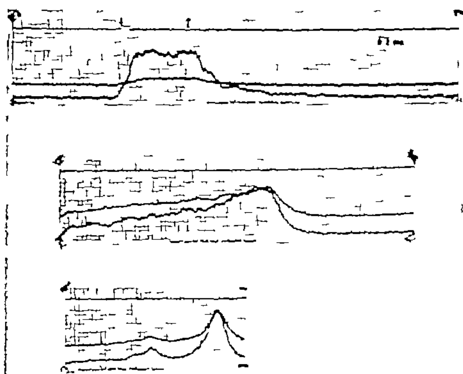


Fig 5 Pat. L. G O (Tbc pulm. sin.)
Curve 1 Radioactive argon was inhaled and measured on both sides The upper curve is from the left lung and the lower from the right Curve 2 and 3 Calibration tests. Both detectors registered argon.

Table II

Patients	Place for measuring the activity	Elimination %/sec.	
		Ethyl-iodide	Argon
H. E. H.	Left lung	4.1	0.7
	Right lung	5 0	0.5
L. G O	Left lung	—	—
	Right lung	7 8	< 0.5
P T., I	Left lung	~ 1.5	—
	Right lung	~ 1 0	—
P T II	Left lung	0.8	—
	Right lung	< 1.0	—
E. A.	Left lung	—	—
	Right lung	3.6	< 0.5
K. K. G	Left lung	—	—
	Right lung	4.0	< 0.5
K. G., II	Left lung	0	—
	Right lung	2.7	—
K. G III	Left lung	2.0	—
	Right lung	3.6	—

IV Inhalation of two gases simultaneously in patients without pronounced lung changes

Both detectors on one lung

As a typical example of the registration of two different gases on one side the curve for patient E. A. (17/7 1960) is reproduced This patient had mild asthma. The detectors were placed as near each other as possible. The distance between the centres of the detectors was about 6 cm.

The curve for argon reaches the maximum somewhat later than that for ethyliodide and afterwards remains horizontal. The curve for ethyliodide reaches the top sooner and this must be due to the fact that ethyliodide is taken up and carried away by the blood before inhalation is completed (fig 7)

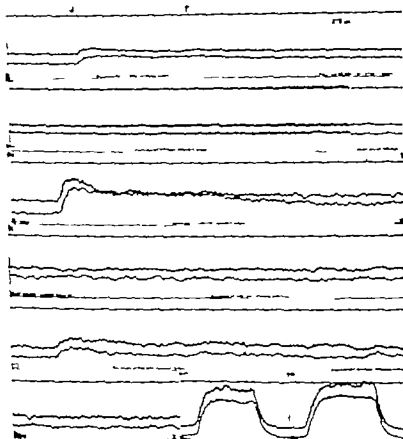


Fig. 9

Fig. 7. Pat. E. A. (Asthma bronchiale + emphysema pulm.)

Curv. 1. Inhalation of gas mixture containing both radioactiv. argon and radioactive ethyliodide. Both detectors were placed on the right lung as near each other as possible. Curve 2 and 3. Continuation of curv. 1. Curv. 4. Calibration tests. Upper detector registered argon. Lower detector registered ethyliodide.

Fig. 8. Pat. K. K. G. (Bronchopneumonia + asthma bronchiale + emphysema pulm.)

Curv. 1. Inhalation of gas mixture containing both radioactive argon and radioactive ethyliodide. Both detectors were placed on the right lung as near each other as possible. Curv. 2 and 3. Continuation of curve 1. Curve 4. Calibration tests. Upper detector registered argon. Lower detector registered ethyliodide.

Fig. 9. Pat. A. O. Asthma bronchiale + status post tbc pulm.)

Curv. 1. Radioactiv. ethyliodide was inhaled and measured on both sides during blocking of the right pulmonary artery; Curve 2. Continuation of curv. 1. Curv. 3. Another inhalation as before, recorded with greater magnification; Curv. 4. Continuation of curv. 3; Curve 5. A third inhalation after removal of the block; Curv. 6. Continuation of curve 5 and calibration tests. Upper curv. line from left lung. Lower curv. line from right lung. Both detectors registered ethyliodide.

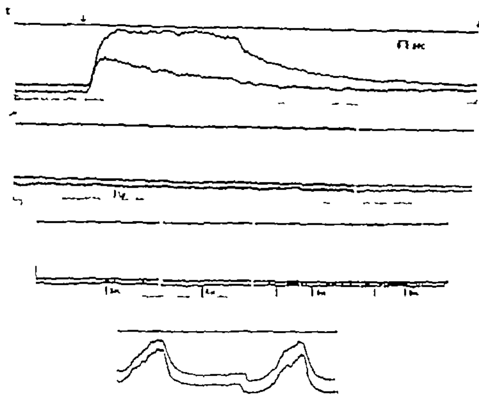


Fig 7

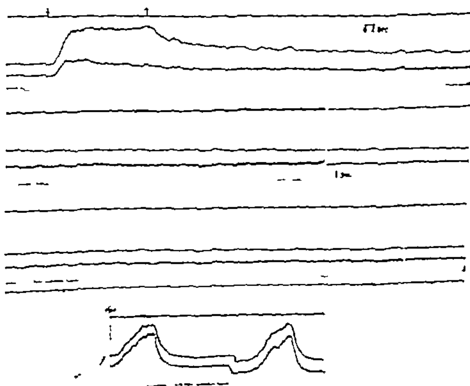


Fig 8

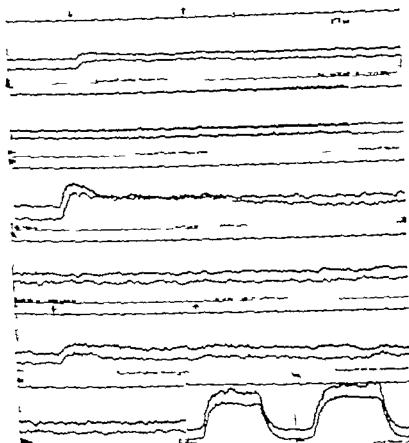


Fig. 9

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Curve 1 Radioactive ethyliodide was inhaled and measured on both sides during blocking of the right pulmonary artery. Curve 2 Continuation of curve 1; Curve 3 Another inhalation as before, recorded with greater magnification. Curve 4 Continuation of curve 3. Curve 5 A third inhalation after removal of the block; Curve 6 Continuation of curve 5 and calibration tests. Upper curve line from left lung. Lower curve line from right lung. Both detectors registered ethyliodide.

held his breath after a maximal inhalation The investigation has demonstrated that gases with different solubilities in water are taken up by the blood at very different rates during the first seconds after inhalation (table II)

It has been shown earlier that gases with very different solubilities in water are eliminated at about the same speed from the blood after intravenous injection If a calculation is made of the amounts of argon and acetylene eliminated in one minute after the injection in some of the patients on whom tests have previously been made (2 b) it is found that the amounts of gas eliminated after one minute expressed as percentages of the amounts injected are only slightly different for argon and acetylene This is set out in the table III.

This relationship may explain the well known fact that retention of a gas can occur in the blood even in the case of gases, which are easily dissolved in water and have a high diffusion rate e. g carbon dioxide. Such a result could be anticipated if differences in the diffusion processes through the lung membranes for the gases used were of such an order that they could almost be disregarded

Further the result may be said to justify the assertion that the diffusion process is not the determining factor as regards gas exchange in the lungs but that ventilation and circulation instead are the decisive factors.

If two gases with greatly different solubilities are made to pass from the blood out to the air in the lungs on one occasion, and on the other occasion in the reverse direction the diffusion processes through the membranes as well as the mixing in the bronchial system in the lungs are alike. The difference observed in our experiments may be explained by taking into consideration the differences in solubilities in water

Ethyliodide is suitable for studies of regional lung function and for the assessment of both ventilation and circulation in different parts of the lungs. Only ventilation can be assessed with argon during studies of regional lung function through inhalation of the gas.

Dyson and his colleagues (3) have used oxygen 15 which has both advantages and disadvantages compared with ethyliodide. Among the advantages must be included the fact that the uptake is exactly the same as that for oxygen. Among the disadvantages is the short life of the isotope (half life about 60 seconds) which means that tests can only be carried out in the vicinity of a cyclotron.

In a work not yet published (Colldahl, Dunér Svanborg) the uptake of ethyliodide has been studied in conjunction with the blocking of the pulmonary artery on one side after the insertion of a catheter Here it was noticed that the uptake of ethyliodide on the side that was blocked was much lower than on the other side, which again was greater than normal during the time the patient held his breath. When the patient began to breathe again the activity dropped more quickly on the side which had been blocked, which shows that unchanged ethyliodide was present in the bronchial system and could be exhaled with the exhaled air (fig 9) After ethyliodide has been taken up in the body however a change occurs so that the full quantity of the gas taken up is not exhaled. How ethyliodide is changed after uptake in the blood is not yet completely known.

One advantage of regional lung function studies with the isotope technique

is that this method of investigation can also be carried out with patients who cannot be bronchoscoped or where separate tube connections with each of the lungs cannot be achieved. This was the case as regards the patient L. G. O., who was quoted as an example.

Summary

1. In earlier reports the simultaneous passage of different gases with different solubilities in water (acetylene, argon, xenon) from the blood to the alveolar air has been studied, especially during the first 20 seconds after the intravenous injection of the gases in saline solution. In this report the simultaneous passage from the alveolar air to the blood has been studied for two gases (ethyliodide and argon) with important differences as regards their solubilities in water.

2. The gases used have been ethyliodide containing ethyliodide with radioactive isotope I^{131} and argon containing the radioactive isotope Ar^{41}.

3. The uptake of ethyliodide in healthy patients and in patients with moderate changes of the lungs is about 5 per cent per second during the first 15 seconds. In patients with very severe changes the uptake is lower.

4. During this period the uptake of argon was not very obvious. A difference in the solubilities in water of these two gases is considered to be the reason for this.

5. The results show difference in the rate of lung passage of the gases from the alveolar spaces to the blood and from the blood to the alveolar spaces. Our earlier reports have shown, that if argon and acetylene are injected intravenously they are eliminated at about the same rate in healthy persons. The solubility of ethyliodide in water is of the same order as the solubilities in water of carbon dioxide and acetylene and differs from those of argon and oxygen.

6. Considering our results in this and earlier investigations (1, 2) with those of Liljestrand and Sahlstedt (9) making use of an isolated frog lung, it seems to be obvious that diffusion is not a limiting factor for the passage of gases through the lungs in the living organism. Instead circulation and ventilation are of decisive importance. An important factor for the rate of uptake of gases in the body through the lungs is the solubility of the gases in the blood. This factor does not seem to be of any great importance for the passage in the opposite direction, when the gases already are dissolved in the blood.

7. Radioactive ethyliodide can be used for studies of the regional lung function, especially during the first 30 seconds after inhalation, in order to study both ventilation and circulation. When using argon, only ventilation can be studied during this initial period through inhalation of the gas.

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One advantage of regional lung function studies with the isotope technique

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A Comparison of Crude Liver Extract and Crystalline Vitamin B_{12} in the Treatment of Pernicious Anemia

By

Poul Bastrup-Madsen

Since the discovery of the anti-anemic properties of vitamin B_{12} it has been a matter of discussion whether liver extract exerts its action in pernicious anemia solely by virtue of its content of vitamin B_{12}, or if it contains essential factors other than vitamin B_{12} and folic acid. Even though it is a commonly accepted clinical experience that a remission can be brought about and maintained by crystalline vitamin B_{12}, it is still an unsolved problem whether some minor alterations which can be corrected only by administration of liver persist in such patients.

In the solution of this problem a comparison of the efficacy of the two substances in producing a complete remission in previously untreated cases of pernicious anemia is of importance. In the first studies after the discovery of vitamin B_{12} the investigators using crystalline vitamin B_{12} isolated from liver found responses comparable to those known from other cases treated with liver extract. The same observation has repeatedly been made after administration of crystalline vitamin B_{12} produced from streptomyces, which is the most commonly used commercial vitamin B_{12} preparation. During the first few years, some uncertainty existed as regards the equal efficiency of the two preparations, but this seemed, at least in many cases, to be merely a question of giving correct doses.

Furthermore, it is necessary to compare the efficacy of vitamin B_{12} and liver extract in producing a neurologic remission in cases of pernicious anemia with nerve involvement. Such an investigation has been carried out in an ideal manner by Ungley who found full equivalency of the two substances in this respect.

The next requirement which must be fulfilled is that crystalline vitamin B_{12} must be able to maintain a hematologic and neurologic remission during long-term treatment. Clinical series (Bastrup-Madsen, Conley et al.) confirm the value of vitamin B_{12} in maintenance therapy

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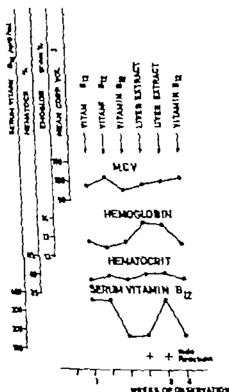


Fig. 1. Case 1. The effect of liver extract on the erythrocyte and serum vitamin B_{12} values in 52-year-old man with pernicious anemia on long-term therapy with vitamin B_{12} given in weekly dose of 60 µg.

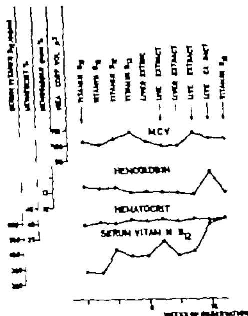


Fig. 2. Case 2. The effect of liver extract on the erythrocyte and serum vitamin B_{12} values in a 53-year-old woman with pernicious anemia on long-term therapy with vitamin B_{12} given in dose of 60 µg every second week.

The patients were seen in our out-patient clinic during the whole period in which they received vitamin B_{12} therapy. Blood samples were always obtained at the same hour, viz. 8—9 a.m. The patients were not fasting. The blood for cell counts was drawn from a vein stabilized with dipotassium ethylenediamine tetra-acetate. At the same time, blood was also withdrawn for vitamin B_{12} assays. After the withdrawal of blood the patient was given an injection of vitamin B_{12} or liver extract. In this way, the blood vitamin B_{12} level was determined after maximum interval from the preceding injection and thus represented the lowest value between two injections.

The hemoglobin level was determined by a photo-electric hemoglobinometer (Hemotest Tesla). Red and white blood cells were counted in a Burger-Türck counting chamber. The volume of packed red cells was determined by means of Wintrobe hematocrit tubes. All blood smears were examined by the author.

Vitamin B_{12} was assayed microbiologically using lactobacillus Leichmannii. The determinations were performed by lic. agro. Sai Chin Van, of the Research Laboratories of Medo-Dumex Ltd., Copenhagen.

The liver preparation was crude liver extract generously supplied by Medo-Dumex.

Normal erythrocyte values for the laboratory of Aarhus Amtssygehus (mean ± standard deviation) are indicated below. The normal series consisted of 20 women and 20 men, whose age ranged from 20 to 40 years. These individuals were in good health, had normal values for reticulocytes, leukocytes, platelets, serum iron and vitamin B_{12}, and had a normal bone marrow smear.

Table I Therapy given before trial with liver extract in 10 cases of megaloblastic anemia due to vitamin B_{12} deficiency (one case with megaloblastic anemia following subtotal gastrectomy (no. 4) and nine with Addisonian pernicious anemia)

Case no.	Age (years)	Sex	Duration of pernicious anemia (years)	Other types of therapy	Duration of vitamin B_{12} therapy (years)	Duration of present vitamin B_{12} dosage schedule (months)	Interval between present doses of 60 μg (weeks)
1	52	M	16	Liver hog stomach	5	12	1
2	53	F	20	Hog stomach	2	6	2
3	68	F	2	—	2	3	2
4	69	M	2	—	2	2	2
5	68	F	27	Liver hog stomach	5	4	3
6	77	F	7	Hog stomach	6	2	4
7	76	M	2	—	2	2	4
8	74	M	2	—	2	3	4
9	74	M	4	—	4	3	4
10	57	M	12	Liver hog stomach	2	12	4

Finally the demonstration whether or not a hematologic remission in patients on long term vitamin B_{12} therapy may be further improved by liver extract must be conclusive.

It is the purpose of this paper to present and analyse pertinent clinical data obtained in a study of patients with pernicious anemia.

Material and methods

The series consisted of 10 patients with megaloblastic anemia due to vitamin B_{12} deficiency (table I) Nine of the patients had Addisonian pernicious anemia and one (case 4) had a megaloblastic anemia following subtotal gastrectomy

In all the patients the nature of the anemia was evidenced by the presence of a macrocytic anemia, megaloblastic bone marrow histamine-fast achlorhydria and a satisfactory reticulocyte response to specific anti-pernicious anemia therapy followed by a rise in the red blood cell counts.

Five of the patients had only received injections of vitamin B_{12} before the trial with liver extract the remaining five had received therapy with other preparations (for either oral or parenteral use, or both) before they were switched on to maintenance therapy with injections of vitamin B_{12}. All the patients had received maintenance therapy with injections of vitamin B_{12} for 2 to 6 years before the trial with liver extract.

At the time when liver extract was given a trial the intervals between the injections of vitamin B_{12} were one week in one case two weeks in three, three weeks in one and four weeks in five cases. Each injection consisted of 60 μg The patients had received maintenance therapy in the dosage mentioned for from 2 to 12 months. Each patient received liver injections at intervals of the same length as had been employed in the vitamin B_{12} injections. The liver extract contained 2 6 μg liver vitamin B_{12} per ml. The dose of liver given in each injection was 4.5 ml crude liver extract. To this amount of liver extract was added so much crystalline vitamin B_{12} that the total content of vitamin B_{12} ("liver plus crystalline vitamin B_{12}") in the injected amount of liver was 60 μg

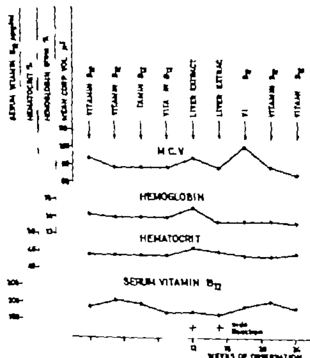


Fig. 5. Case 5. The effect of liver extract on the erythrocyte and serum vitamin B_{12} values in 68-year-old woman with pernicious anemia on long-term therapy with vitamin B_{12} given in dose of 60 μg every third week.

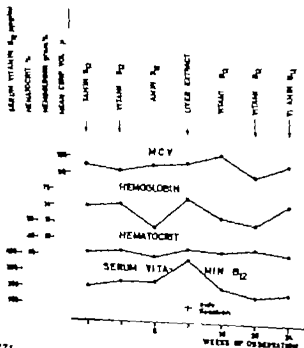


Fig. 6. Case 6. The effect of liver extract on the erythrocyte and serum vitamin B_{12} values in 77-year-old woman with pernicious anemia on long-term therapy with vitamin B_{12} given in dose of 60 μg every fourth week.

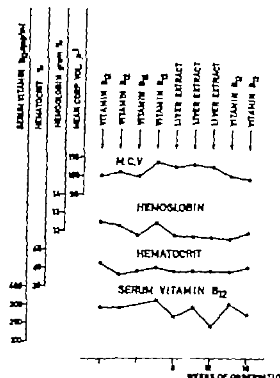


Fig 3. Case 3 The effect of liver extract on the erythrocyte and serum vitamin B_{12} values in a 68-year-old woman with pernicious anemia on long-term therapy with vitamin B_{12} given in a dose of 60 µg every second week.

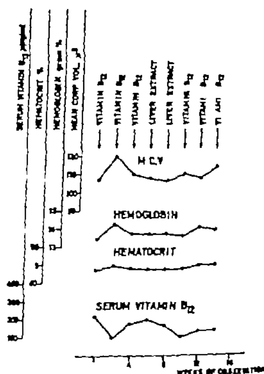


Fig 4 Case 4 The effect of liver extract on the erythrocyte and serum vitamin B_{12} values in a 69-year-old man with megaloblastic anemia following subtotal gastrectomy performed 14 years previously The patient was on long-term therapy with vitamin B_{12} given in a dose of 60 µg every second week.

Hemoglobin (g per 100 ml blood) males, 15.5 ± 0 75 females, 13.5 + 0.75.
Erythrocytes (mill./µl) males 5 00 ± 0.25 females, 4.25 ± 0.25.
Hematocrit (vol.) males, 47 ± 2 5 females, 42 ± 2 5.
Mean corpuscular volume (M C.V µ³) 97 ± 5.
Mean corpuscular hemoglobin concentration (M C.H.C., g per 100 ml) 33 ± 1 5

Normal serum vitamin B₁₂ levels (Meco-Dumex Research Laboratories) ranged from 131 to 1 600 µµg/ml and averaged 399 µµg/ml in a series of 40 healthy individuals with normal erythrocyte values and normal bone marrow The reproducibility of the analysis was examined by performing a total of 17 analyses on the same deep-frozen sample of serum on various days within a period of 3 months. This study revealed that at the mean value found, 488 µµg/ml, the standard devia

Results

The influence of injection of crude liver extract on the hematologic remission and on the serum vitamin B_{12} level in patients with pernicious anemia on maintenance therapy with vitamin B_{12} is analysed in figs. 1—10 The values for hemoglobin hematocrit, M C.V and vitamin B_{12} are shown in these figures, whereas the red cell counts are not stated, as they appear indirectly from the values for M C V which are calculated from the erythrocyte and hematocrit values.

Evaluation of erythrocyte values In this paper "the erythrocyte values" signify hemoglobin, red cell count hematocrit and M C.V In all cases, these values were within the normal range for our

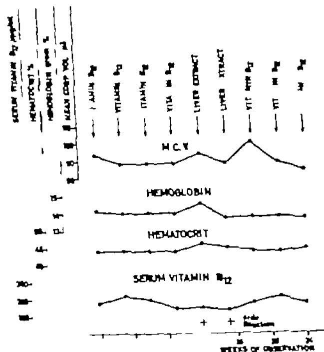


Fig. 5. Case 5. The effect of liver extract on the erythrocyte and serum vitamin B_{12} values in 60-year-old woman with pernicious anemia on long-term therapy with vitamin B_{12} given in a dose of 60 µg every third week.

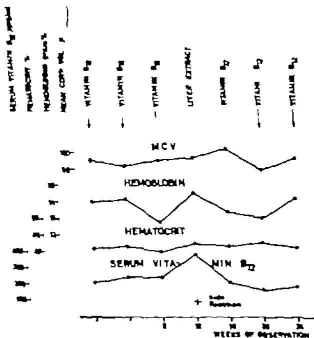


Fig. 6. Case 6. The effect of liver extract on the erythrocyte and serum vitamin B_{12} values in 77-year-old woman with pernicious anemia on long-term therapy with vitamin B_{12} given in dose of 60 µg every fourth week.

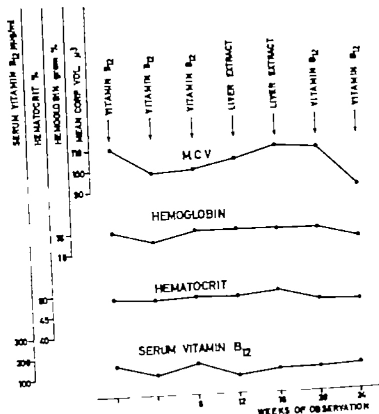


Fig 7 Case 7 The effect of liver extract on the erythrocyt and serum vitamin B_{12} values in a 76-year-old man with pernicious anemia on long-term therapy with vitamin B_{12} given in a dose of 60 μg every fourth week.

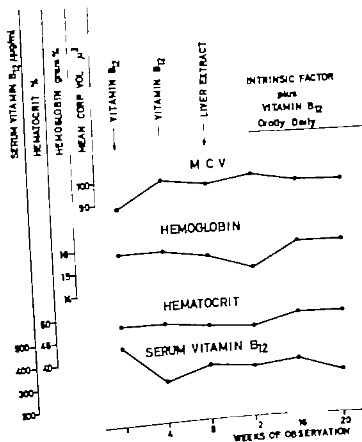


Fig 8. Case 8. The effect of liver extract on the erythrocyt and serum vitamin B_{12} values in 4-year-old man with pernicious anemia on long-term therapy with vitamin B_{12} given in dose of 60 μg every fourth week.

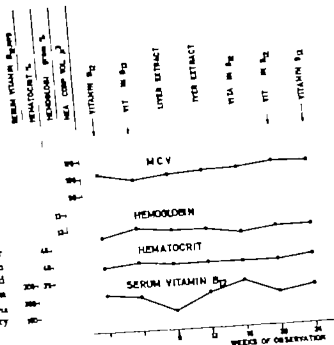


Fig 9. Case 9. The effect of liver extract on the erythrocyte and serum vitamin B_{12} values in 74-year-old man with pernicious anemia on long-term therapy with vitamin B_{12} given in dose of 60 µg every fourth week.

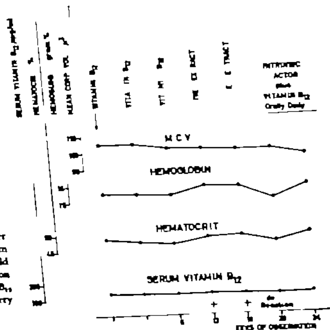


Fig 10. Case 10. The effect of liver extract on the erythrocyte and serum vitamin B_{12} values in 57-year-old man with pernicious anemia on long-term therapy with vitamin B_{12} given in dose of 60 µg every fourth week.

laboratory and were fairly constant within the period of observation. It is of special interest to note that none of the patients revealed changes in the M.C.V. after the liver injections. In most cases insignificant fluctuations were observed in the hemoglobin and hematocrit values, but experience shows that such fluctuations always occur in an out-patient series. The two cases (Nos. 1 and 2) in which changes in these values occurred in relation to the change-over from vitamin B_{12} to liver extract therapy will be considered in some detail in the discussion.

Evaluation of serum vitamin B_{12} values. The curves show that the serum vitamin B_{12} concentration fluctuated slightly more than the erythrocyte values, as might be expected when using a biological method with the accuracy and sensitivity concerned. In view of the efficiency of the method, the serum vitamin B_{12} level must be said to have remained fairly constant within a certain range in each case during the various therapeutic procedures. The importance of the changes in the serum vitamin B_{12} level which occurred in cases 1, 2 and 9 will also be considered in the discussion.

Side reactions to liver extract injections were seen in four cases. Fever (40 C) and pruritus of one day's duration developed after each of the two injections given in case 1. Fever also occurred after each of the two injections given in case 4; two or three hours after the second injection urticaria and severe edema of duration developed in this patient. Fever occurred on the day of injection in case 6 and followed each of the two injections of liver extract in case 10. No discomfort was experienced in any of the 10 patients after vitamin B_{12} injections.

Discussion

In spite of the efficiency of small doses of vitamin B_{12} in producing a remission it has been suggested by some authors that this vitamin is not the only substance lacking in pernicious anemia. Larsen found that some macrocytosis still persisted in patients treated with crystalline vitamin B_{12}, and stated that only liver extract was able to induce normocytosis. Owren claimed that a reduced prothrombin concentration was more rapidly and completely restored by liver extract than by vitamin B_{12}. Even though most observers have been unable to confirm either of these two observations, some doubt still exists concerning the complete efficiency of vitamin B_{12} in pernicious anemia, although the data collected to an increasing extent seem to support the suggestion that liver extracts are acting solely by their content of vitamin B_{12}.

Vitamin B_{12} (cyanocobalamin) was isolated from liver tissue in 1948. In 1949 this vitamin was crystallized from streptomyces. The first trials of the effect of cyanocobalamin in pernicious anemia were made with the substance crystallized from liver, while vitamin B_{12} isolated from streptomyces has later become the drug most commonly used. When the term crystalline vitamin B_{12} is used in this paper without further comments "streptomycin vitamin B_{12}" is meant.

In dealing with the problem of equivalency of vitamin B_{12} and liver extract two questions must be considered. First, the possibility that vitamin B_{12} from liver may have other properties than streptomycin vitamin B_{12} must be kept in mind. Secondly, there is the question whether liver contains essential factors other than vitamin B_{12} and folic acid.

Vitamin B_{12} occurs naturally bound to peptide or protein (Lester Smith). An

observation in favour of the theory that vitamin B_{12} as it occurs in liver tissue may have other properties than crystalline vitamin B_{12} was reported by Reizenstein and Nyberg, who compared the intestinal absorption of radioactivity after oral administration of liver containing labelled vitamin B_{12} with that of crystalline radio-vitamin solutions. These experiments seemed to show that both normal individuals and patients with pernicious anemia absorbed more of the liver vitamin B_{12}. This observation need not necessarily imply that there is a difference in the action of injected liver and streptomycin vitamin B_{12}.

Another observation was made by Sheely et al., who claimed that, when given as liver extract, vitamin B_{12} circulated in the blood in a higher proportion as the bound form and was retained for a longer period in the organism. However these authors thought that the most likely explanation of this observation was that vitamin B_{12} was absorbed more slowly when it was injected as liver extract.

In a clinical investigation in which liver extract and crystalline vitamin B_{12} are compared, there will be some practical difficulties involved in investigating separately the two problems mentioned (i.e., the possible difference between the properties of the two kinds of vitamin B_{12} and the presence of a still unidentified factor in the liver). If such an investigation shows that there is no difference in the action of crystalline vitamin B_{12} and liver extract, these two problems must be said to be solved in common.

It was the aims of the present investigation to examine whether liver extract might produce a further improvement of the erythrocyte values in patients with pernicious anemia treated with crystalline vitamin B_{12}, whether liver would be able to reduce the size of the erythrocytes in these patients and whether an alteration in the serum vitamin B_{12} level could be produced.

In these experiments, care was taken that the injections of liver extract were given at the same intervals as the injections of crystalline vitamin B_{12}, and that the patients were given the same total dose of vitamin B_{12} (liver plus streptomycin vitamin B_{12}) as they had received during the preceding maintenance therapy. The difference in the treatment was then that some part of the vitamin B_{12} originated from the liver and the presence of the liver extract itself.

Ten patients with megaloblastic anemia due to vitamin B_{12} deficiency were investigated. These patients had been on maintenance therapy with injections of crystalline vitamin B_{12} for from 2 to 6 years.

The figures illustrating the experiments contain a few values from the preceding maintenance therapy and a few from the therapy given afterwards, in order that random fluctuations should not appear as probable results of liver treatment.

In all the cases the values for hemoglobin, red blood cells, hematocrit, M.C.V. and serum vitamin B_{12} were within the normal limits of our laboratories. As far as M.C.V. is concerned, it must be mentioned that many of the patients had values higher than those stated as normal in the literature (for example, by Wintrobe). However for some reason or other the normal values for M.C.V. are somewhat higher in our laboratories than indicated in the literature. In all the cases the values for M.C.V. were within our normal range, with due consideration of the errors of analysis. Thus all the patients seemed to be in

hematologic remission without macrocytosis while they were receiving maintenance therapy with vitamin B_{12}. No neurologic symptoms or signs had developed during this therapy.

Even though the erythrocyte values were within the normal range, this need not necessarily imply that the remissions were complete. It was therefore examined whether injections of liver extract were able to improve the erythrocyte, hemoglobin and hematocrit values and to reduce the size of the erythrocytes. To liver extract containing a certain amount of "liver vitamin B_{12}" was added the usual form of crystalline vitamin B_{12} ("streptomycin vitamin B_{12}") so that the total amount of vitamin B_{12} which the patient received corresponded to the amount he had received during the preceding maintenance therapy. In this way the patients acted as their own controls.

In connection with the assessment of the *erythrocyte values* two cases must be discussed in detail.

In *case 1* (fig. 1) the hemoglobin concentration was about 13 g per 100 ml during the vitamin B_{12} therapy; one week after the first liver injection it had increased to about 14 g. If this had been an actual increase referable to an improved hematologic remission caused by the liver injection, a simultaneous fall in the M.C.V. should have been expected. However, the M.C.V. did not show any change after the liver injection. Moreover, it will be seen from the figure that on the day when the first liver injection was given (i.e. immediately before the injection) the hemoglobin concentration was also about 14 g, while it had fallen to about 13 g one week after the second injection. The two values of 14 g must therefore be regarded as accidental fluctuations unrelated to the liver injections.

In *case 2* (fig. 2) an increase in the hemoglobin level from about 13 to 14 g per 100 ml occurred after the fourth liver injection. As the hemoglobin concentration after the fifth injection was again about 13 g, this single value of 14 g must also be considered to be quite accidental.

In the remaining eight patients, no changes occurred in the erythrocyte values after injection of liver extract. Normocytosis was present in all cases, and the M.C.V. was not reduced after the institution of liver therapy.

It must therefore be said that the experiments did not reveal any evidence suggesting that liver extract contains vitamin B_{12} in a form which is capable of improving the hematologic status in patients with pernicious anemia who have previously been treated with crystalline vitamin B_{12}, or that liver extract should contain other substances which can improve the remission which has been obtained by vitamin B_{12}.

In the evaluation of the *serum vitamin B_{12} level* three cases must be considered in some detail.

In *case 1* (fig. 1) an increase in the vitamin B_{12} concentration from about 200 to about 400 $\mu\mu$g/ml occurred one week after the first liver injection. If this increase was more than apparent, suggesting that it would be possible to obtain a higher serum vitamin B_{12} concentration by administration of liver extract, it should also have persisted after the second injection, but at that time the concentration had again fallen to about 200 $\mu\mu$g/ml. Accordingly, the increase was scarcely due to the liver injection, but must be ascribed to uncertainty in the analysis. Further evidence in support of the latter assumption is the fact that fluctuations

of this order of magnitude were also observed during the vitamin B_{12} therapy before the trial with liver

In *case 9* (fig 9) injection of liver extract was followed by an increase from about 100 to 250 $\mu\mu g$/ml. It is seen from the graph that the serum vitamin B_{12} level was fairly constant, about 200 $\mu\mu g$/ml, apart from this single low value immedi ately after the first liver injection. Ac cordingly the increase is scarcely due to the liver injection, but is probably refer able to an erroneous determination on the day of the first liver injection.

Case 2 (fig. 2) was that in which the largest number of liver injections (5) was given. After the fourth and fifth injections the serum vitamin B_{12} level increased from about 400 to about 600 $\mu\mu g$/ml. However this increase did not exceed what had in some of the other cases been justifiably described as accidental variations, although it cannot straight away be rejected as only apparent, since it was observed in two successive determinations. The question is then if the liver may act as a depot after a sufficient number of injections. The series is not large enough for such a conclusion, but it must be pointed out that the possibility exists.

The remaining seven patients did not reveal any changes in the serum vitamin B_{12} level after injection of liver extract.

Thus, the present experiments do not definitely confirm the theory advanced by Sheely et al. that a slow absorption of vitamin B_{12} from the liver extract resulting in prolonged action should occur On the other hand, it may justifiably be concluded that if liver extract has prolonged action, this is so slight that it is without clinical importance However as only part of the vitamin B_{12} which was administered in each injection was "liver vitamin B_{12}" the present experiments do not provide an answer to the question whether equal parts of "liver vitamin B_{12}" and crystalline vitamin B_{12} may give rise to different serum concentrations. If the dosage level of 60 μg employed should have been given as "liver vitamin B_{12}" an amount of 25 ml of crude liver extract should have been injected. In the present experiments in which the dose of liver extract injected was 5 ml — a dose level in common use and rarely exceeded in a single therapeutic injection — the serum vitamin B_{12} concentration was the same as during treatment with crystalline vitamin B_{12}. It does not seem to have been investigated whether liver extract administered in amounts equivalent to 60 μg of "liver vitamin B_{12}" per injection would be able, by a prolonged action, to maintain a higher vitamin B_{12} level in the blood. However as experimental evidence does not suggest that "liver vitamin B_{12}" has a biological action different from that of crystalline vitamin B_{12}, the problem is of little importance, as it would be unreasonable to use so large doses as would then be required, because these doses would give rise to considerable local discomfort and also to a greatly increased risk of sensitization. If a preparation with a prolonged action is desirable, other methods of production are available.

Fever followed injection of liver extract in four cases, and in one of these extensive urticaria and edema developed. This frequency of side reactions is in agreement with the author experience in a larger series of patients with pernicious anemia treated with liver extract. Fever occurs relatively often after liver injection, whereas allergic reactions are rarer but when they do occur they are very unpleasant and may lead to collapse.

The patients often complain of tenderness at the site of injection persisting for a couple of days. None of these untoward reactions are seen after injection of vitamin B_{12}. As it cannot be demonstrated that liver has any therapeutic advantages over vitamin B_{12}, it will be reasonable to prefer the latter in order to avoid the side reactions. In addition since vitamin B_{12} can be manufactured at a lower cost, there are actually no reasons for employing liver extract in the treatment of pernicious anemia.

Conclusions

In agreement with other reports, the present study suggests that injections of crystalline vitamin B_{12} are adequate in the treatment of pernicious anemia.

The investigation did not reveal any evidence in favour of the assumption that crude liver extract contains other factors or vitamin B_{12} in a form which is capable of producing further improvements in the hematologic status in patients who were on maintenance therapy with crystalline vitamin B_{12} such patients did not show any increase in the hemoglobin level and the erythrocyte values or any decrease in the M.C.V. The serum vitamin B_{12} concentration was the same when crystalline vitamin B_{12} or crude liver extract was used. A prolonged action of liver extract suggested by other investigators could not be demonstrated but owing to the experimental conditions employed, the theory could not be disproved. However it must be pointed out that on injection of the usual therapeutic doses a possible prolonged action by liver extract is of no clinical importance.

It is reasonable to assume that liver preparations are active by virtue of their content of vitamin B_{12}. Liver therapy has therefore no therapeutic advantages over treatment with crystalline vitamin B_{12}. On the basis of the investigation it may even be concluded that injections of crystalline vitamin B_{12} are preferable to liver injections, because unpleasant allergic side reactions developed in four of the 10 patients after liver injections, while no discomforts were observed after injections of crystalline vitamin B_{12}.

Summary

Ten patients with megaloblastic anemia due to vitamin B_{12} deficiency who had been on maintenance therapy with crystalline vitamin B_{12} for from 2 to 6 years were given a series of injections of crude liver extract. The study did not reveal any evidence suggesting that liver extract should result in a better hematologic status, reduce the size of the erythrocytes, or maintain a higher serum vitamin B_{12} concentration than crystalline vitamin B_{12}. In view of the side reactions observed in four of the patients after the liver injections it is concluded that injection of crystalline vitamin B_{12} should be preferred in the treatment of pernicious anemia.

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Coronary Disease in a Group of Norwegian Tram Drivers

By

Knut Joachim Berg and Anders Melkild

There are few investigations illustrating the significance of mental factors in the development of coronary disease. Observations suggest that there is a connection between mental stress and coronary disease, partly in provoking the acute attack (Russek and Zohman 1958) and partly in respect of long term development of coronary disease (Ryle and Russel 1949 Weiss et al. 1957 Wolf 1959)

Individuals subjected to dissimilar mental stress usually also differ as regards physical activity smoking and eating habits and personality Detailed investigations of occupations in which the incidence of coronary disease is especially high may be of interest for the discussion of the etiology of the disease.

The starting point for the present investigation was the fact that a strikingly large number of tram drivers were admitted to the Central Hospital in Trondheim with myocardial infarction We have tried to ascertain the incidence of coronary disease in this occupation group and attempted to assess the significance of certain etiological factors in the development of the disease. We have in particular tried to elucidate the significance of the stress factor

Material

The present investigation includes all regular tram drivers in Trondheim who were in employment on Jan. 1st 1959 and all those who had been in active employment in the course of the 5 years preceding this date.

Sixty-seven drivers satisfied the above criteria, of whom 5 were more than 70 years old. Two of these 5 had died and no case history could be found, and it was not possible to investigate the other 3 satisfactorily The material thus consists of 62 tram drivers, all less than 70 years old. Three were dead, but detailed hospital records were available

The remaining 59 drivers have all been examined by the authors When examined, 44 were in active employment, 8 had retirement pensions (retirement age 68 years) and 7 disablement pensions. None had quit their job for other reasons in the course of the last 5 years.

The average age was 59.0 years (37—69 years) and the average length of employment in the tram company was 37 1 years with 19.8 years as regular tram drivers.

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Table I. Average age and average length of employment of the 62 tram drivers

	No. of cases	Aver age age (yrs.)	Average no. yrs. em-ployed	Average no. yrs. as regular drivers
Dead or pen-sioned	18	65.8	41.2	29.1
Active tram drivers	44	56.3	35.4	15.9
Total	62	59.0	37.1	19.8

Table II. Age distribution of the drivers with coronary disease

Age (11—59)	No. of cases investi gated	Infarc tion	Angina pectoris
60—69	41	8	5
50—59	10	2	1
< 50	11	0	0
Total	62	10	6

Practically all had driven trams periodically before being promoted to regular tram drivers, thus giving a somewhat longer total driving period.

Method

The investigation included a detailed family history questions about working habits, extra work, financial position, social life, physical activity and eating and smoking habits. Special notice was taken of nervous symptoms and clinical symptoms of coronary disease and their possible accentuation while driving especially during periods with difficult working conditions.

A complete clinical examination was carried out supplemented by ophthalmoscopy ECG examination with 12 leads, and also an ECG after exercise unless this was contraindicated because of cardiac disease.

Laboratory investigations included analysis of urine, examination of hemoglobin, erythrocyte sedimentation rate and serum cholesterol (Lund et al. 1961) Saturated and unsaturated fatty acids in serum were investigated with the method of Notevarp and Gyrin (1960)

In this investigation coronary disease means angina pectoris or previous myocardial infarction. The diagnosis was based on the usual criteria (WHO 1959) in particular on the following

For the diagnosis of *angina pectoris* a typical case history was required, with rapid relief of pain by nitroglycerine. In addition emphasis was put on ECG changes, either left ventricular hypertrophy without other known cause or signs of coronary insufficiency at rest or after exercise.

The diagnosis of *myocardial infarction* was based on the case history clinical findings and ECG findings at the present investigation and on previous admissions to hospital.

Results

Definite signs of coronary disease were found in 16 of the 62 tram drivers, or 25.8 per cent. Ten of these had myocardial infarction, 8 of whom were hospitalised and detailed case histories were available. In all these cases the diagnosis of myocardial infarction was certain. Two of the hospitalised patients died as a result of their infarction. One died immediately after admission with typical symptoms of infarction. ECG was not recorded, but autopsy showed a fresh myocardial infarction. The other patient who died had a posterior wall infarction. Two patients were *not admitted to hospital* Both had typical case histories, and ECG showed an infarction in one case and right bundle branch block of the Wilson type in the other

Six drivers had angina pectoris. Five of them had ECG changes. In one patient with a normal resting ECG exercise was not carried out because of dyspnoea and pronounced angina pectoris.

The age distribution of the material is shown in table II

The average age when disease appeared at 37 1 years and on the time of investigation 63 1 years.

Six drivers without coronary disease were considered to have organic heart disease of other natures (hypertonic heart disease 3, aortic stenosis 1 and cardiosclerosis 2) Of 31 tram drivers over 50 years, a total of 22 thus suffered from organic heart disease.

Eight drivers with coronary disease, 6 with previous infarction, were in active employment at the time of the investigation. Their average age was 60.4 years and their average period of service after development of coronary disease was 60 months.

All the drivers with coronary disease were over 50 years old. In this age group there were 16 men with coronary disease and 35 others, both groups with almost the same average age and average period of driving The most important clinical findings and the results of laboratory tests for these two groups, excluding the 3 patients who had died, are shown in table III

Hypertension means BP ≶ 160/100 or a diastolic BP ≶ 110, measured three times at a single examination, with the patient laying down after rest Overweight means normal weight + 15 per cent (N tvig 1956) The upper limit for normal serum cholesterol with the method used is about 300 mg (Lund et al. 1961)

Overweight and hypertension were found more frequently than normal in both groups, but were equally frequent in both of them.

Nervous symptoms such as fear tremor sleeplessness and functional troubles involving the heart and other organs were definitely more common in the group with coronary disease. Some of these complaints, however were certainly secondary to the coronary disease.

Table III Clinical findings and laboratory tests in drivers with coronary disease and in other drivers over 50 years

	With c. d. (total 14)	Without c. d. (total 34)
Overweight	8	15
Hypertension	5	13
Nervous symptoms	6	3
Cardiac enlargement	5	6
Syst. murmur ≥ grade II	8	10
Serum cholesterol, ≥ 300 mg	4	11
B S R, ≥ 20 mm	7	3
HB, < 13 g/100 ml	1	5
Proteinuria	0	3

The laboratory tests showed that raised serum cholesterol was about equally frequent in both groups. In 22 of the drivers over 50 years a closer analysis of the total fatty acids and poly-unsaturated fatty acids in serum (Notevarp and Cyvin 1960) was carried out. Eleven had symptoms of coronary disease, 8 of them having had myocardial infarction. The average values of the saturated fatty acids were highest in the group with coronary disease, while the polyunsaturated fatty acids were equally high in both groups (table IV)

The anamnestic investigation gave information about cardiovascular disease (coronary disease sudden death) in the family of 5 of the 16 tram drivers with coronary disease and in 8 of the other 45 investigated.

The figures concerning tobacco consumption, caloric intake and physical activity (table V) are certainly influenced

Table I Average age and average length of employment of the 62 tram drivers

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Dead or pensioned	18	65.8	41.2	29.1
Active tram drivers	44	56.3	35.4	15.9
Total	62	59.0	37.1	19.8

Table II Age distribution of the drivers with coronary disease

Age (41—69)	No. of cases investigated	Infarction	Angina pectoris
60—69	41	8	5
50—59	10	2	1
< 50	11	0	0
Total	62	10	6

Practically all had driven trams periodically before being promoted to regular tram drivers, thus giving a somewhat longer total driving period.

Method

The investigation included a detailed family history, questions about working habits, extra work, financial position, social life, physical activity and eating and smoking habits. Special notice was taken of nervous symptoms and clinical symptoms of coronary disease and their possible accentuation while driving, especially during periods with difficult working conditions.

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Results

Definite signs of coronary disease were found in 16 of the 62 tram drivers, or 25.8 per cent. Ten of these had myocardial infarction, 8 of whom were hospitalised and detailed case histories were available. In all these cases the diagnosis of myocardial infarction was certain. Two of the hospitalised patients died as a result of their infarction. One died immediately after admission with typical symptoms of infarction. ECG was not recorded, but autopsy showed a fresh myocardial infarction. The other patient who died had a posterior wall infarction. Two patients were not admitted to hospital. Both had typical case histories, and ECG showed an infarction in one case and right bundle branch block of the Wilson type in the other.

Six drivers had angina pectoris. Five of them had ECG changes. In one patient with a normal resting ECG exercise was not carried out because of dyspnoea and pronounced angina pectoris.

The age distribution of the material is shown in table II.

The average age when disease appeared was 57.1 years and on the time of investigation 63.1 years.

Six drivers without coronary disease were considered to have organic heart disease of other natures (hypertonic heart disease 3, aortic stenosis 1 and cardiosclerosis 2). Of 51 tram drivers over 50 years, a total of 22 thus suffered from organic heart disease.

Eight drivers with coronary disease, 6 with previous infarction, were in active employment at the time of the investigation. Their average age was 60.4 years and their average period of service after development of coronary disease was 60 months.

All the drivers with coronary disease were over 50 years old. In this age group there were 16 men with coronary disease and 53 others, both groups with almost the same average age and average period of driving. The most important clinical findings and the results of laboratory tests for these two groups, exluding the 3 patients who had died, are shown in table III.

Table III. Clinical findings and laboratory tests in drivers with coronary disease and in other drivers over 50 years

	With c. d. (total 14)	Without c. d. (total 54)
Overweight	8	15
Hypertension	5	13
Nervous symptoms	8	3
Cardiac enlargement	5	6
Syst. murmur, ≧ grade II	9	10
Serum cholesterol, ≧ 300 mg %	4	11
B S R, ≧ 20 mm	7	3
HB, < 13 g/100 ml	1	5
Proteinuria	0	3

Hypertension means BP ≧ 160/100 or a diastolic BP ≧ 110 measured three times at a single examination, with the patient laying down after rest. Overweight means normal weight + 15 per cent (Natvig 1956). The upper limit for normal serum cholesterol with the method used is about 300 mg % (Lund et al. 1961).

Overweight and hypertension were found more frequently than normal in both groups, but were equally frequent in both of them.

Nervous symptoms such as fear, tremor, sleeplessness and functional troubles involving the heart and other organs were definitely more common in the group with coronary disease. Some of these complaints, however, were certainly secondary to the coronary disease.

The laboratory tests showed that raised serum cholesterol was about equally frequent in both groups. In 22 of the drivers over 50 years a closer analysis of the total fatty acids and poly-unsaturated fatty acids in serum (Notevarp and Oyvin 1960) was carried out. Eleven had symptoms of coronary disease, 8 of them having had myocardial infarction. The average values of the saturated fatty acids were highest in the group with coronary disease, while the polyunsaturated fatty acids were equally high in both groups (table IV).

The anamnestic investigation gave information about cardiovascular disease (coronary disease, sudden death) in the family of 5 of the 16 tram drivers with coronary disease and in 8 of the other 45 investigated.

The figures concerning tobacco consumption, caloric intake and physical activity (table V) are certainly influenced

Table IV Distribution of saturated and poly-unsaturated fatty acids in drivers with and without coronary disease

	No.	Aver age age	Serum chol. mg %	Total saturated fatty acids mg %	Total poly unsaturated fatty acids mg %	Total fatty acids mg %
With coronary disease	11	62.5 —	267 —	385 (240—582)	149 (115—204)	534 (356—726)
Without coronary disease	11	64.2 —	276 —	324 (235—474)	146 (102—198)	470 (371—673)

Table V Anamnestic information regarding nicotine consumption, caloric intake and physical activity apart from work

	Nicotine consumption (60)			Caloric intake (58)			Physical activity apart from work (61)		
	L	M	S–N	L	M	S	L	M	S
Tram drivers with coronary disease, premorbid period	4	8	3	3	12	0	5	3	8
Tram drivers over 50 years without coronary disease	4	19	11	5	19	8	21	6	8
Tram drivers under 50 years	5	5	1	1	10	0	5	3	2

L = large M = medium S = small N = none.

by subjective prejudices. In the group with coronary disease we have tried to assess the condition in the premorbid period.

Both tobacco consumption and caloric intake are perhaps higher in the group with coronary disease than in the group without. The caloric and fat intake hardly vary from the average values in the general population

There was little physical activity during work. Physical activity apart from work was difficult to assess. Most drivers had ample exercise in their free time, including those with coronary disease in the pre morbid period. The majority had their own houses and gardens, very few owned a car Many used bicycles or took part in sports.

Mental stress Most of the drivers, especially in the group over 50 years, said spontaneously that the tram driving was tiring and involved repeated nervous strain. The conditions are most difficult during autumn and winter when the steep streets become slippery because of fallen leaves, snow and ice. The statistics show an increased number of accidents during these seasons. Strikingly many tram drivers stated that they had more symptoms of angina pectoris during periods with difficult driving conditions, others developed nervous symptoms. It might be mentioned that several drivers noticed the first symptoms of coronary disease after a fire in which many trams were destroyed, and after which the new trams were replaced by old models for a time.

Discussion

In this study of 62 tram drivers (average age 59.0 years) the prevalence of coronary disease was 25.8 per cent.

This is a far higher figure than usually reported in large American (Boas and Epstein 1954, Chapman et al. 1957 Stamler et al. 1960) and English (Brown et al. 1957 Morris et al. 1952) investigations where the prevalence of coronary disease in men in corresponding age groups usually varies between 5 and 10 per cent. There are no corresponding Norwegian statistics, but recent investigations from Oslo City Hospitals (Westlund 1961) suggest that the incidence of infarction in men is slightly lower in Oslo than in USA.

With the method of investigation we have used, it is reasonable to expect a higher prevalence of coronary disease than in investigations based on hospital cases. The present investigation included all those under 70 years who had ceased work in the course of the past 5 years. No man began working as tram driver after coronary disease had started, and changing over to other occupations in earlier years had very infrequently happened. The average period of employment, 37.1 years, also shows the stability of this occupation group.

It is therefore not likely that the observed prevalence of coronary disease depends on chance in selection and method.

The cause of this high prevalence of coronary disease is not known. It is possible that constitutional factors have been of importance. Morris (1960) thus found a difference in constitution between tram drivers and tram conductors in London. Family history did not indicate that inheritance had been of any great importance in our material.

The distribution of saturated and unsaturated fatty acids was slightly different in the two groups (table IV). The tests were, however, in some cases made a few months after an acute infarction, and in many cases after a change in diet and may therefore not be representative. Reliable figures of the body weight in the premorbid period were not available. The high incidence of adiposity indicates a relatively large caloric intake in proportion to the physical activity. Several epidemiological investigations indicate that there is a preponderance of coronary disease in the physically inactive (Breslow and Buell 1960 Brown et al. 1957 Morris et al. 1953). Morris et al. (1953) found a considerably higher incidence of coronary disease in tram drivers than in conductors employed by London Bus Transport.

More recently Rosenman and Friedman (1958) showed that the conductors in the centre of London with the greatest traffic, had a higher incidence of coronary disease than the tram drivers in the outskirts of the city.

Friedman och Rosenman (1959) have shown that patients with coronary disease often have a special behavior pattern and a characteristic way of reacting to mental stress. Several investigators have demonstrated changes in the serum lipids (Friedman et al. 1958, Hammarsten et al. 1957 Wertlake et al. 1958) and blood coagulation (Friedman et al. 1958) in persons subjected to mental stress. Russek (1960) has recently investigated American doctors in the age group 40—70 years, and found that the incidence of coronary disease increased in proportion to the stress factor in each occupational group.

The question of whether one occupational group is exposed to greater mental stress than another must be the object

of subjective judgement. Reactions to external factors will also to a great extent depend on the constitution of the individual. There are several reasons to believe that the work of the tram drivers entails unusually severe forms of mental stress especially during autumn and winter. It is, however, not known whether or not there is a causal relationship between the high incidence of coronary disease and the occupational stress in the present material.

In Norway there are no definite rules as to whether tram drivers with coronary disease should be permitted to continue their job. Eight of 16 tram drivers with coronary disease were still working actively. No accidents that could be connected with cardiac attacks had occurred during work. One of the drivers developed myocardial infarction while driving the tram, but he was able to stop the tram safely.

Summary

The authors have investigated the prevalence of coronary disease in 62 tram drivers in Trondheim. The average age was 59.0 years and the average period of employment in the same job 37.1 years. All those who had stopped active work in the course of the past 5 years were included in the investigation. Fourty four were working, 15 were pensioned and 3 were dead.

Complete cardiological investigation with supplementary laboratory tests was carried out, and special emphasis was put on obtaining an accurate past history in all cases.

Sixteen of 62 tram drivers had coronary disease, 10 of whom myocardial infarction and 6 angina pectoris. Six had other forms of heart disease. The prevalence of coronary disease was 25.8 per cent for all those investigated and 18.2 per cent for those still working.

Hypertension was found frequently and to the same degree in drivers with coronary disease as in the other drivers. Signs of nervousness were most common in the group with coronary disease.

The total physical activity of the tram drivers was below the average. Their diet and smoking habits did not seem to differ from the average.

The drivers were exposed to severe mental stress, and many of them had to report sick in periods with difficult driving conditions, on account of increasing cardiac and nervous symptoms.

The possible significance of the stress factor is discussed in more detail.

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Changes in the Coagulation System Following Major Surgical Operations

By

O. Egeberg

The postoperative period represents increased risks for thrombotic diseases. Although this has long been recognized, data concerning changes in the blood coagulation system after surgical procedures are still relatively scarce. Such data ought to give some of the best opportunities for analyzing the possible relationship between coagulation and thrombosis.

The present study intended to evaluate if any definite pattern of changes in clotting factor levels could be found postoperatively. To attain this, most of the known clotting factors had to be controlled. Because of technical difficulties, this could, however, only be done for a small number of patients, and for a limited period of time for each patient.

The same patients were investigated with particular regard to fibrinogen and fibrinogen interactions, the results for which are reported in a separate paper (Godal 1962).

Material

The investigations were done on patients admitted to Surgical Department B, Rikshospitalet, Oslo. Blood samples were collected in the days before surgery and during the postoperative period. Seven patients were selected for the study: 4 men and 3 women. Table I gives the principal data for these patients. One of the patients, (No. 6) with stomach cancer died on the tenth postoperative day from generalized peritonitis, and in a second patient with the same disease (No. 7) the cancer was too extended to be removed.

Anticoagulants were not given, and thrombotic complications were not observed.

The blood samples were taken in the morning: control samples on the last days before the operation, and a limited number of samples during the next 8–23 days.

Plasma samples for assaying clotting factors. Plasma was prepared as "silicone" citrated plasma. Blood was taken by puncture of an antecubital vein with a siliconized needle. The first 2—3 ml of blood were discarded, and 9 parts of blood were then collected directly into chilled siliconized glass tubes containing one part of 3.1 g per cent sodium

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Fig. 1 Results of coagulation tests and platelet counts for the patients No. 1 to 4

method, as performed in the present investigations, is sensitive also to variations in Stuart Prower factor (factor X).

Fibrinogen was measured according to Jacobsson (1955) with the modification of Blombäck & Blombäck (1956).

Results

The results of the investigations are shown in fig. 1 and fig. 2. Blood transfusions to the patients were very limited, (see figs. 1 and 2) and the transfusions cannot explain the main results as to variations in clotting factor levels.

1. *Platelets.* In case Nos. 1, 2, 3 and 4 is seen a decrease in platelet counts during the first 1 to 4 postoperative days, followed in case Nos. 1, 2 and 3 by an increase. Case Nos. 5 and 6 show an increase of the platelet counts without any decreased values in the first days. The especially high platelet counts in No. 5 must be ascribed to the splenectomy in this case. In case No. 7, in which only an explorative laparotomy was done, the platelet number did not change significantly.

2. *Global tests for plasma coagulation.* The changes in the cephalin time and thromboplastin time were for most of the cases relatively small, and showed no marked

Table I. Data for the seven cases studied

Patient	Sex	Age	Diagnosis	Type of surgery	Result
1. J. R.	M	48	Ca. coli	Resectio coli	No complications
2. J. S.	M	68	Ca. coli	Resectio coli	No complications
3. S. O.	F	33	Tbc. pulm.	Resectio segm. pulm.	No complications
4. H. L.	F	20	Tbc. pulm.	Resectio segm. pulm.	No complications
5. R. T.	F	65	Tumor ventr. Diabetes mellitus	Gastrectomia et splenectomia	No complications
6. O. R.	M	67	Ca. ventr.	Gastrectomi	Radical operation, but mors on the 10th post-op. day in peritonitis
7. A. J.	M	78	Ca. ventr.	Laparatomia explorativa	Radical operation proved impossible

citrate dihydrate solution. After mixing the blood samples were centrifuged at 2,500 rpm. (max. g ca. 1 800) for 30 min. at 4° C. Plasma was pipetted off with a siliconized pipette, and every sample was distributed into a number of siliconized glass tubes, immediately frozen and kept at −20° C until assaying. The same technic was used for preparation and handling of the different "silicone" citrated factor-deficient plasmas used as substrates in the assays.

Testing of the plasma samples was done several weeks after their preparation, to minimise the errors due to differences in duration of storage.

When testing the different clotting factors, a series of test samples from the different dates from one patient were thawed simultaneously and the assaying of a particular factor could then be done with the same batch of reagents.

The hematocrit value was determined for each blood sample. The changes in hematocrit could not, however, explain the main observed changes in the concentration of the different clotting factors. The listed values have not been corrected for the hematocrit changes.

Blood samples for platelet count. One ml of blood for platelet count was taken by a two ml syringe, already containing one ml of 3.1 g per cent sodium citrate dihydrate solution. This mixture was then diluted with 18 ml of the citrate solution to give a 1/20 final dilution for the platelet count.

Cephalin suspension was prepared according to the method of Hjort et al. (1955).

Dilution fluids. Two different fluids for dilution of plasma were used, I and II (Hjort et al. 1955). A plasma was first diluted in citrated saline (fluid I) to 20 times the final concentration, then the final dilution 1/20 was done in buffered citrated saline (fluid II).

Human tissue thromboplastin. A buffered saline suspension prepared and stored according to Hjort (1957) was used.

Methods

Platelet count was determined according to the method of Nygaard (1933).

Cephalin time was estimated according to Egeberg (1961).

Thromboplastin time with human brain thromboplastin was determined according to Waaler (1959), equal amounts of the thromboplastin suspension and of dilution fluid II being added to the test plasma before the incubation.

Antihemophilic A factor (AHA = factor VIII), antihemophilic B factor (AHB = factor IX) and antihemophilic C factor (AHC = plasma thromboplastin antecedent = PTA = factor XI) were assayed with one-stage cephalin systems using plasma from patients with severe deficiencies as substrates (Egeberg 1961). The assaying was ordinarily done in a 1/20 or 1/50 dilution of the test plasmas.

Proaccelerin (factor V) was estimated by the method of Owren (1947).

Proconvertin (factor VII) was estimated by the method of Nas (1952).

Prothrombin (factor II) was estimated with the method of Hjort et al. (1955). This

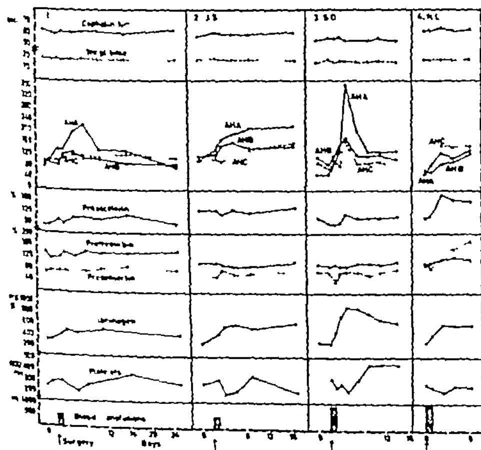


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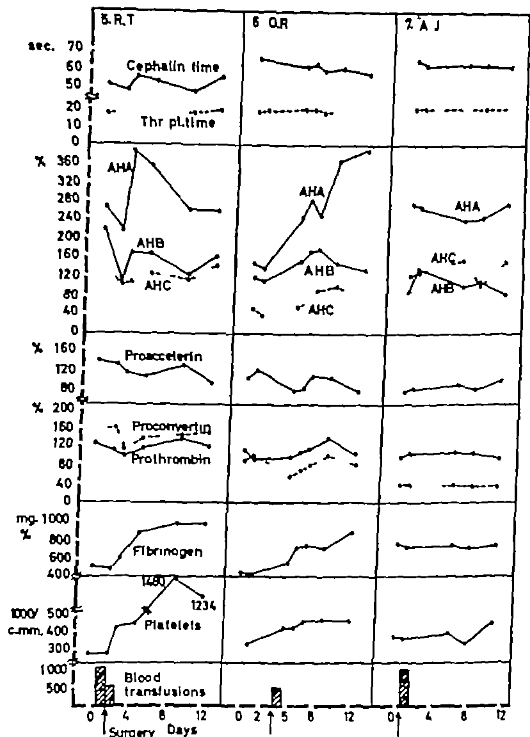


Fig 2 Results of coagulation tests and platelet counts for the patients N 5 to 7

general trend. Plasma from patient No 5 with diabetes mellitus and cancer ventriculi, had before the operation a short cephalin time (around 50 sec. against normal range of 57 to 64 sec.) which persisted after the operation (see fig 2) In only one patient, No 6 who died of peritonitis one day after the last blood sample, was there seen a marked shortening of the cephalin time. Also for this pa-

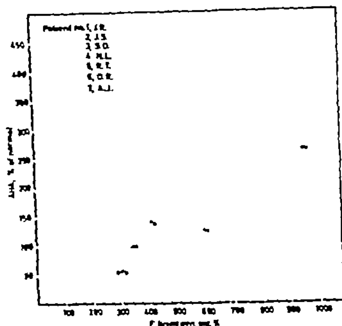


Fig. 3. Corresponding values of AHA and fibrinogen measured in the plasma samples from the 7 patients.

tient the thromboplastin time was quite stable.

3. *Antihemophilic A factor* Plasma samples from patient No. 7 with cancer ventriculi and extensive metastases, showed before the operation extremely high AHA values, ca. 280 per cent of normal. No extensive surgery was undertaken on this patient, and the postoperative AHA values differed little from the preoperative levels. In all the other cases, the measured AHA level increased markedly after surgery (see figs. 1 and 2) both in cases (Nos. 3 and 6) with high preoperative values and in the cases with lower levels beforehand (Nos. 1 2, 3 and 4) The highest values were reached in case No. 3 after segmental pulmonary resection, in the case No 5 after gastrectomy and in No. 6 after gastrectomy and splenectomy with levels up to between 360 and 400 per cent of normal. The maximal AHA values were observed from 1 to 15 days after surgery

4 *Antihemophilic B and C factors.* The results show a tendency to measured increased AHB and AHC values on the first days after extensive surgery but the tendency was less marked than for AHA.

5. *Proaccelerin, proconvertin prothrombin.* Both increased and decreased values were found postoperatively and no uniform trend can be discerned.

6. *Fibrinogen.* On all the cases, except No. 7 there is seen a significant increase of the fibrinogen concentration after surgery These changes are discussed in detail in a separate paper (Godal 1962)

In fig 3 it is seen that there is a marked tendency to positive co-variation between the measured levels of AHA and fibrinogen.

Discussion

The changes in platelet number observed after surgery are in accordance with findings of other workers (Hueck 1925 Warren 1933 Pepper 1960) who

have showed a tendency to thrombocytopenia in the first 1 to 3 postoperative days, followed by a tendency to thrombocythemia with a maximum about 1 to 2 weeks after the operation.

Previous investigations in which global clotting tests were made in the postoperative period have given differing and partly contradictory results Shapiro et al (1942) found shortened prothrombin times postoperatively whereas Gardikas et al. (1959) could not observe any definite change. The present results are consistent with the latter report

Gardikas et al on examining 200 patients, found a slight average decrease of the plasma recalcification time postoperatively with the shortest time on the 5th postoperative day Fergulio et al (1960) in an investigation of 15 cases, found prolonged whole blood clotting times on the third postoperative day and shortened times on the 7th day while the recalcification times seemed to be shortened on both days. In the present study the plasma cephalin time was used as a global test for the intrinsic coagulation activity No uniform change was discerned by this test. These results illustrate how difficult it is to reveal abnormal high values of certain coagulation factors by such global tests.

The assaying of proaccelerin proconvertin and prothrombin did not show any characteristic pattern. Definite conclusions as to the behavior of these factors postoperatively would need a bigger material.

The investigations on intrinsic clotting factors showed one striking finding in all cases with extensive surgery namely a postoperative increase in measured AHA. The increase was correlated with an increase in the fibrinogen levels. This finding gives additional support to the hypothesis that there is a closer connection between the levels of AHA and fibrinogen in human plasma (Egeberg 1962 a & b) when using the present methods as specific measures of these factors.

The high AHA levels in the postoperative period together with the increased fibrinogen concentration and increased platelet count, indicate a potential hypercoagulable state, which could possibly contribute to the high incidence of thrombosis after surgery

As to AHB and AHC, the results also seem to show a tendency to higher postoperative levels in five of the six cases of extensive surgery Some of this measured increase in AHB and AHC might possibly be ascribed to an effect on the test system caused by extremely high AHA values of the test plasma. This, however cannot be the whole explanation. The results strongly indicate that some more general clot accelerating principles are released into the blood in the days following surgery Such thromboplastic material may give an accelerating effect in the assays of several factors This would then account for findings such as the marked changes in the levels of different clotting factors found in the presented cases after surgery on the lungs

Summary

From 7 patients blood samples were taken before and after major surgery Platelet counts and different clotting tests were done. Six of the patients underwent extensive surgery because of abdominal cancer or lung tuberculosis. On one patient with abdominal cancer only an explorative laparotomy was done The patients were followed with postoperative blood samples from 8 to 23 days after surgery

Some of the cases showed a decrease in platelet count in the first 1 to 4 days after the operation, and most of the cases an increased count about 1—2 weeks after the operation. This is in accordance with the findings of other investigators.

Only moderate and indefinite changes were found in plasma cephalin times and plasma thromboplastin times in most of the cases.

The measured variations in proacceler in (factor V), proconvertin (factor VII) and prothrombin (factor II) showed no definite general pattern.

Antihemophilic A factor (AHA = factor VIII) showed a marked postoperative increase in the cases after extensive surgery. The maximal AHA values were observed from 1 to 15 days after surgery. The high AHA levels were correlated with high fibrinogen concentrations.

For antihemophilic B factor (AHB = factor IX) and antihemophilic C factor (AHC = factor XI) there seemed also to be tendency to higher levels in the first days after surgery. It cannot be answered to what extent these results are an expression of non-specificity of the assay methods, caused by the liberation of clot accelerating principles postoperatively affecting coagulation methods of the type used.

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As to AHB and AHC, the results also seem to show a tendency to higher postoperative levels in five of the six cases of extensive surgery Some of this measured increase in AHB and AHC might possibly be ascribed to an effect on the test system caused by extremely high AHA values of the test plasma. This, however cannot be the whole explanation The results strongly indicate that some more general clot accelerating principles are released into the blood in the days following surgery Such thromboplastic material may give an accelerating effect in the assays of several factors. This would then account for findings such as the marked changes in the levels of different clotting factors found in the presented cases after surgery on the lungs.

Summary

From 7 patients blood samples were taken before and after major surgery Platelet counts and different clotting tests were done Six of the patients underwent extensive surgery because of abdominal cancer or lung tuberculosis. On one patient with abdominal cancer only an explorative laparotomy was done. The patients were followed with postoperative blood samples from 8 to 23 days after surgery

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Quantitative and Qualitative Changes in Fibrinogen Following Major Surgical Operations

By

H. C. Godal

A transient increase in the plasma content of fibrinogen is regularly found following major surgical operations (Warren, Amdur, Belko & Baker 1950, Godal & Fichera 1961). In addition, evidence has been brought forward that fibrinogen is also qualitatively altered under these circumstances. Thus, "fibrinogen B" assumed to be an intermediate between fibrinogen and fibrin (Lyons 1945) is markedly increased during the first post operative days (Cummine & Lyons 1948, Ryan 1951). Further another fibrinogen-like substance, the heparin-precipitable fraction described by Smith & von Korff (1957) is similarly increased following surgery (Godal & Fichera 1961).

In this work, the plasma levels of clottable material and of fibrinogen-like substances were closely followed during the post-operative period in a series of patients. Simultaneously variations in the thrombin-fibrinogen reaction were studied. The close correlation between fibrinogen concentration and heparin tolerance demonstrated previously (Godal & Fichera 1961) was confirmed. Further the plasma levels of "fibrinogen B" and of the heparin-precipitable fraction varied in parallel with that of total clottable protein.

Submitted for publication November 10, 1961

Material and methods

Seven patients, admitted to the surgical department B, Rikshospitalet, Oslo, were selected for the study. A detailed description of the material is published elsewhere (Egeberg 1962). None of the patients were treated with anticoagulants and no thromboembolic complications occurred.

The blood samples were drawn in the morning, using siliconized equipment. The samples were immediately cooled (except for preparation of serum, and for study of the heparin-precipitable fraction) and centrifuged within 30 minutes after withdrawal of the blood.

Beta naphthol solution. A two per cent solution in 50 per cent ethanol was used.

Cryofibrinogen was assayed according to the method given by Kalbfleisch & Bird (1960).

Fibrinogen was determined by the method of Jacobsson (1955) as modified by Blombäck & Blombäck (1957).

Plasma fibrinogen (gm/100 ml)

0.90 0.75 0.60 0.45 0.30

O.R. R.T A.J. S.O. H.L. J.S J.R

Surgery

2 4 6 8 10 12 14 16 18 20 22 24

Days

Fig. 1 The plasma fibrinogen content in seven patients following major surgical operations.

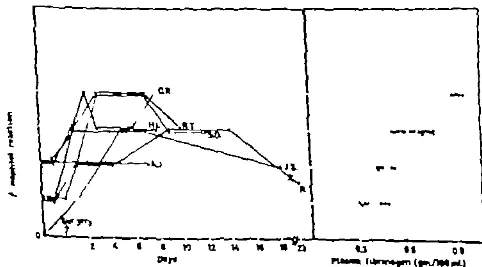


Fig. 2 The plasma "fibrinogen B" content in seven patients following major surgical operations and the "fibrinogen B" content as related to total clottable protein.

Fibrinogen B was determined according to the method of Lyons (Cummine & Lyons 1948) and the results graded as proposed by Smith et al. (1951)

Heat resistance of fibrinogen was tested as follows 0.5 ml aliquots of platelet-poor citrated plasma, in test tubes (glass) were placed in a water bath of 50° C, and the temperature allowed to rise at about 0.2° C per minute The time elapsing until incipient flocculation was recorded. Further the appearance of the precipitate was noted.

Heparin thrombin time (*HTT*) was assayed according to the technic described previously (Godal 1961 b)

Heparin-precipitable fraction was assayed as described previously (Godal & Fichera 1961)

Plasma fraction I was prepared according to the method VI of Cohn et al. (1946) and used immediately

Platelet-poor citrated plasma was obtained as described earlier (Godal 1960) The samples were stored deep frozen in stoppered, siliconized tubes.

Serum was obtained by clotting whole blood for two hours at 37° C while stirring After centrifugation, the serum was pipetted off and stored as the plasma.

Thrombin. A bovine preparation, "Topostasin" Hoffmann La Roche Basel, Switzerland, was used

Thrombin time The following clotting system was used

0.2 ml platelet poor plasma,
0.2 ml saline,
0.2 ml thrombin.

The strength of the thrombin solution was selected to give a thrombin time of normal plasma of approximately 60 seconds. The clotting mixture was incubated for three minutes at 37 C prior to the addition of thrombin

Other materials and methods used in this work have been described previously (Godal 1960 1961 a)

Results

1. THE PLASMA CONCENTRATION OF FIBRINOGEN AND OF FIBRINOGEN LIKE SUBSTANCES

A Fibrinogen. The variations in fibrinogen concentrations are illustrated in fig 1

The observed marked increase in plasma fibrinogen between the third and ninth postoperative day is in accordance with earlier reports (Warren et al. 1950 Godal & Fichera 1961) Marked individual variations were evident, and in one patient (A. J) no change was observed. In most cases, the preoperative values were doubled, and in one patient (S O) an even greater increase was found (figs. 1 and 7)

B "Fibrinogen B" This fibrinogen-like substance also increased markedly during the first postoperative days. Further a close correlation between the concentration of fibrinogen and that of "fibrinogen B was observed (fig 2)

These observations are consistent with previous findings of Smith et al. (1951) When compared with fig 1 it is seen that fibrinogen and "fibrinogen B" paralleled each other in the individual cases and again the patient A. J failed to respond.

C Heparin-precipitable fraction (*HPF*) This fraction, partly clottable, precipitates from heparinized plasma in the cold (Smith & von Korff 1957) It is increased in diverse pathological conditions (Smith 1958) and also during the first postoperative days, returning to normal prior to fibrinogen itself (Godal & Fichera 1961)

This last observation was confirmed (fig 3) Consequently low fibrinogen concentrations were invariably found together with low HPF values, whereas the correlation was poorer for medium and high fibrinogen concentrations.

D Cryofibrinogen. In the presence of high levels of fibrinogen, a cold-precipitable, clottable protein has repeatedly been demonstrated (Korst & Kratochvil 1955 Kalbfleisch & Bird 1960 Campbell, Hammack & Frommeyer 1959) It

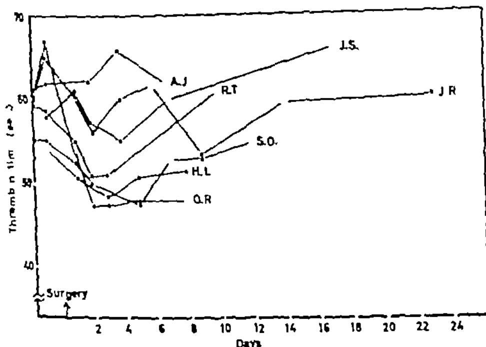


Fig. 4. The thrombin time of plasma, following major surgical operations.

Table I. Heat resistance of fibrinogen in plasma samples from the patient S. O. as compared with normal controls. The plasma samples from the patient are enumerated successively (For details, see methods)

Sample	Fibrinogen concentration (mg 100 ml)	Time until incipient precipitation	Temp. (0 C) at which precipitation occurred
1	314	4 00	51.5
2	504	4 15	52.0
3	503	2' 30'	51.0
4	766	2 45	51.0
5	928	2' 15	51.0
6	914	2 40'	51.0
9	645	3' 05	51.5
Normal plasmas			
1	288	5 00	52.0
2	324	5 30''	52.0
3	318	4 45	52.0

Table II. Heat resistance of fibrinogen in normal plasma, as related to the fibrinogen concentration. Normal plasma fraction I (n.d.) was dissolved in normal, platelet-poor citrated plasma, giving final fibrinogen concentration of 1 106 mg 100 ml. Thereafter the mixture was serially diluted with plasma. (For details see methods)

Sample	Fibrinogen concentration (mg 100 ml)	Time until incipient precipitation	Temp. (0 C) which precipitation started
1	1 106	3 45	51.5
2	698	4 30	52.0
3	481	4 30'	52.0
4	318	4 45	52.0

C HTT as correlated with the concentrations of fibrinogen, fibrinogen B' and HPF

Previously it was shown that plasma fibrinogen and heparin tolerance varied

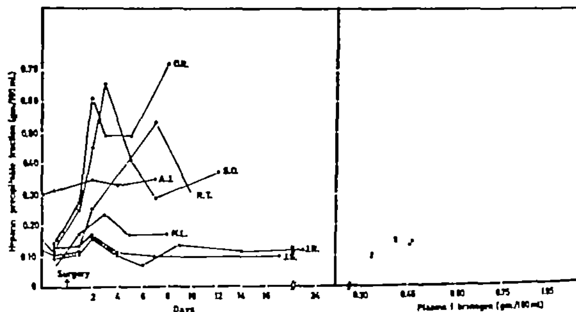


Fig 5. The heparin-precipitable fraction (HPF) and its relation to fibrinogen following major surgical operations.

was therefore of interest to see whether such cold insoluble fibrinogen occurred also postoperatively

However in no instance could a precipitate be observed on incubating citrated plasma at + 4 C for 24 hours. Therefore, even very high fibrinogen values do not necessarily render fibrinogen insoluble in the cold.

II. HEAT RESISTANCE OF FIBRINOGEN

Previous observations (Godal, 1961 c) suggested that a positive fibrinogen B test was not solely due to an increased fibrinogen concentration but also to greater instability of the fibrinogen. It was therefore attempted to discover such an instability by exposing plasma to moist heat

As seen in table I flocculation occurred earlier in plasma obtained from blood drawn in the postoperative state than in normal plasma. In addition, the precipitated fibrinogen was simultaneously altered, and in some instances resembled a clot. These phenomena are not solely due to higher postoperative fibrinogen levels, as judged from the results obtained with normal plasma containing various concentrations of fibrinogen (plasma fraction I) (table II)

III. COAGULATION OF FIBRINOGEN BY THROMBIN

A. Thrombin time As far as the author is aware, no information is available from the literature concerning the effect of surgery on the thrombin time of plasma.

As is evident from fig 4 the thrombin time is moderately shortened during the first days after operation.

B Heparin thrombin time (HTT) HTT becomes gradually shortened after operation, in accordance with earlier observations (Holger Madsen & Schiøler 1959/1960 Godal & Fichera 1961) (fig 5) Again, great individual variations were observed, and again the patient A. J failed to demonstrate significant variations (see also fig 7)

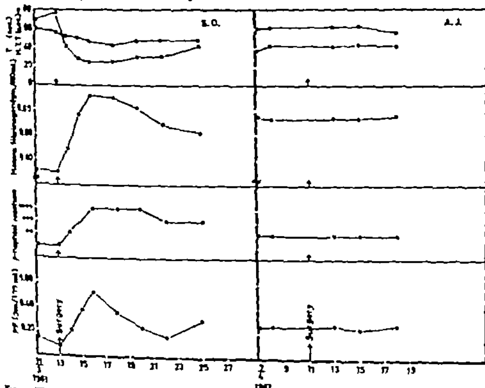


Fig. Thrombin time (TT), heparin thrombin time (HTT), fibrinogen, "fibrinogen B" and heparin-precipitable fraction (HPF) in two patients, S. O. and A. J., following major surgical operations.

Discussion

This report has confirmed earlier observations as to the effect of surgery on the plasma fibrinogen level, and on the heparin tolerance, as judged from the heparin thrombin time. Also the positive correlations between the content of total clottable protein and that of various fibrinogen-like substances are in accordance with previous observations (Smith Rosenfeld & Shinowara 1951, Godal & Fichera 1961, Godal 1962). Such correlations might lead to the concept that the concentrations of such fibrinogen-like substances ar solely due to quantitative changes in fibrinogen. As to "fibrinogen B" this view is held by Smith et al. (1951). However the matter is probably not so simple. Thus, there is no exact parallelism between the concentration of total clottable protein and that of fibrinogen-like substances. Very high fibrinogen concentrations could be present without evidence of cryofibrinogenemia. Further plasma components other than fibrinogen seem to be of importance for the solubility of fibrinogen under various conditions (Godal 1962). Supported by the observations on heat resistance of fibrinogen, there are good reasons to believe that th occurrence of the so-called fibrinogen-like substances is du to a greater tendency of fibrinogen to aggregate, and that this tendency is closely elated to the concentration of clottable protein. However the existence of qualitatively different fibrinogens can at present neither be asserted nor be ex cluded.

Fig 5 The heparin thrombin time (HTT) of plasma following major surgical operations

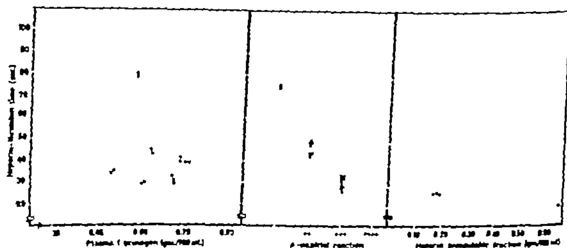


Fig 6. The heparin thrombin time (HTT) as correlated with the concentrations of fibrinogen, "fibrinogen B" and heparin-precipitable fraction (HPF) following major surgical operations.

in parallel during postoperative states, whereas the correlation between heparin tolerance and HPF was less consistent (Godal & Fichera 1961)

These observations were confirmed (fig 6) As is seen, the correlation between fibrinogen concentration and heparin tolerance is also good in individual cases (fig 7) As would be expected, a close correlation between heparin tolerance and fibrinogen B" content in plasma was also demonstrated (fig 6)

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Postural Circulatory Changes at Rest and during Exercise in Patients with Funnel Chest, with Special Reference to Factors Affecting the Stroke Volume

By

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Funnel chest is a congenital thoracic deformity where the body of the sternum and the adjacent parts of the ribs are dislocated backwards, giving rise to an abnormally narrow sternovertebral distance. In pronounced cases the lower part of the sternum may be concavely deformed. In this more severe type of funnel chest, to which the cases in this report belong, the heart is usually displaced to the left and has a flattened appearance on X ray. The deformity can occur familiarly (31, 5). The pathogenesis is unknown, even though several theories have been published (5, 32, 25). The lung volumes may be somewhat reduced but serious impairment of respiratory function at rest seems to be very rare (30, 7). Concerning the influence on cardiac function contradictory opinions are reported. This is to some extent explained by the fact that relatively few studies with heart catheterization in patients with funnel chest have hitherto been published. Most reports comprise only a small series and the hemodynamic data are often incomplete. Some investigators (39, 25) are of the opinion that the deformity in moderate or severe cases may result in profound disturbances of cardiac function. Lyons et al. (27) report one case, investigated with heart catheterization where an increased end-diastolic pressure (12 mm Hg) was found in the right ventricle. The pressure curve was of the type found in cases of constrictive pericarditis. They conclude that this indicates a compressive effect on the right ventricle by the chest deformity. Wachtel et al. (39), Lester (25) and Howard (20) are also of the opinion that a marked funnel chest can restrict the diastolic filling of the heart in a way similar to constrictive pericarditis. However such a mechanism has not been definitely proved. Ravitch (33) has described a case of severe funnel chest with atrial fibrillation, increased pressures in the lesser circu-

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The stability of fibrinogen is probably of importance for maintenance of a normal blood circulation and a normal hemostasis mechanism. An increased tendency of fibrinogen to aggregate or to precipitate might possibly per se favor the initiation and development of a thrombus. Since the instability of fibrinogen increases in parallel with the fibrinogen concentration, and since, moreover the concentration of antihemophilia A factor has been found to vary closely parallel to fibrinogen following surgery (Egeberg 1962), a real hypercoagulability might strengthen this tendency. On the other hand, an increased tendency of fibrinogen to aggregate might under certain circumstances lead to or be associated with a bleeding tendency as observed in patients with suffering from cancer of the prostate combined with cryofibrinogenemia (Kalbfleisch & Bird 1960). In addition simultaneous occurrence of vascular occlusion and bleeding tendency combined with an increased tendency of fibrinogen to aggregate, has occasionally been observed (Henstell & Feinstein 1957, Campbell, Hammack & Frommeyer 1959). Henstell & Kligermann (1958) believed that complexing or coprecipitation of abnormal plasma proteins with factors necessary for coagulation of blood might explain the dual occurrence of a hemorrhagic and thrombotic abnormality. Further work is necessary to elucidate these complex problems.

Summary

A study of fibrinogen and fibrinogen-like substances, their relation to each other and to the coagulability of fibrinogen by thrombin was carried out in seven patients undergoing major surgical operations.

A close relationship was found to exist between fibrinogen and the fibrinogen-like substances, i. e. "fibrinogen B" and the heparin precipitable fraction. Further a similar relationship between fibrinogen concentration and heparin tolerance, as judged from the heparin thrombin time, was observed.

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occasionally had noncharacteristic precordial pain, hich had no relation to physical ac tivity However none of the patients had any history of cardiac decompensation or revealed signs of heart disease.

Fifteen patients have been studied with right heart catheterization. Eleven of these cases were studied at rest and during exercise both in the sitting and supine position. Cases no. 2, 4, 8 and 13 were studied only in the supine position. In cases no. 7 8 and 15 angiocardiography was performed.

Methods

Detailed descriptions of methods as pplied in our laboratory have been reported for *ECG* and *orthostatic test* by Holmgren et al. (14 15) and for *spirometric examination* by Holmgren (12)

The physical orking capacity (PWC) was determined in sitting as well as in supine position according to Sjöstrand (36) and Wahlund (40) on an electrically braked bicycle ergometer (17) The PWC_{170} is defined as the work intensity in kpm per minute, which the subject could perform at pulse rate of 170 beats per minute. The work load was increased stepwise every 6 min. until pulse rate of about 170 was obtained. Using the approximately linear relationship between pulse rate and work load, the value of the PWC_{170} is obtained by inter or extrapolation. A relati steady state was defined as being present if the pulse rate did not increase more than 10 beats from the second to the sixth minute

The methods for determination of *total amount of hemoglobin* (THb) and *blood volume* ha been the same as earlier reported by Holmgren et al. (15) Duplicate determinations were made a an interval of one day During the period of this investigation the standard error of single determination amounted to 5 per cent for both the THb and the blood olume.

The *heart volume* was determined in the prone position according to the method de scribed by Larsson and Kjellberg (23) and Kjellberg et al (21) The standard error of single determination amounts to 4 per cent in cases with normal position of the heart. I some patients with funnel chest, where the heart was displaced and flattened, it was difficult to outline the borders and the method has not been used in these cases.

Right heart catheterization The technique used in the laboratory has recently been de scribed by Bevegård et al. (2) The brachial artery was cannulated by a percutaneous technique according to Seldinger (33) For right heart catheterization a double-lumen catheter was used. The reference level for ero pressure in the supine position was taken at 5 cm below the highest level of the fourth rib. In the sitting position the insertion of the fourth rib at the sternum was taken as a reference level for zero pressure Cardiac out put was measured according to the direct Fick method. The expired air was analysed according to Haldane and Priestly (10)

Blood gas analysis. O_2-saturation and hemoglobin concentration were measured spectrophotometrically (18) With this technique, the standard error of single determination of cardiac output amounts to 8.2 per cent t rest and 5.2 per cent during exercise. For stroke volume these values are 8.6 and 6.8 per cent respectively (18, 19)

The calculation of *mechanical fficiency* has been described lsewhere (2)

Procedure during heart catheterization

Eleven patients were studied with heart catheterization at rest and during exercise both in the sitting and supine position. The general procedure has recently been described in detail (2)

After the catheters had been introduced cardiac output and blood pressures were measured at rest in the supine position, and then after pproximately 6 minutes rest, sitting on the bicycle ergometer This was immediately followed by determination during exercise at tw progressive work loads. Cases no 7 and 12 performed only one work load. Case no 3 performed two work loads in sitting position, but only one in supine position. When the investigation in the sitting position had been completed, the patients rested for t least 30 minutes in the supine position. After this time cardiac output and pressures were again measured in the supine position t rest and also during exercise on the same work loads as in the sitting position.

The work loads were chosen with the

lation and a low cardiac output. The hemodynamics were normalized after operative treatment In connection with this report he also reviews several cases from the literature, where there are indications of cardiac embarrassment that may be attributed to this deformity He is of the opinion, that adequate evidence has accumulated to show that cardiac disturbances are not all rare in cases with funnel chest. Lindskog and Felton (26) also report a case where the pressure in the right atrium was elevated (22 mm Hg) and cardiac output somewhat low The hemodynamics however were essentially normalized after digitalization, which speaks against mechanical factors such as pressure from the chest wall, as a reasonable cause of the disturbance in their case.

A large series of patients with this deformity studied with heart catheterization in the supine position, has been published by Fabricius et al. (7) Twenty six cases were studied at rest and four cases also after exercise. However cardiac output was only determined in some cases. In four of their cases organic heart lesions were found (2 cases with ventricular septal defect one case with a patent ductus arteriosus and one with aortic stenosis) All the other cases showed essentially normal hemodynamic conditions

Wachtel et al (39) have studied the circulation in two cases also during exercise. The results, however can not be evaluated as no data are reported. A decreased physical exercise tolerance is reported to occur (24 20) This opinion is however not based upon objective measurement and it has not been analyzed whether a decreased physical working capacity is caused by cardiac or respiratory factors. An extensive functional analysis has been carried out by Bergh and Berglund (1) In 14 cases the stroke volume has been determined in the supine position at rest as well as during exercise. Five cases have also been examined after surgical correction and in some of these the stroke volume and physical working capacity had increased.

In our laboratory investigation of cardiac and pulmonary cases routinely includes determination of the physical exercise tolerance. It was observed that in cases with funnel chest the effect developed at a pulse rate of 170 beats per minute was higher in supine than in a sitting position, whereas there is generally no difference in normal individuals. Since then the effect of body position on the circulation at rest and during exercise in normals has been studied (2) The purpose of the present investigation has been to find the hemodynamic explanation for the influence of body position on the physical working capacity in cases with funnel chest It was also supposed that such a study should give information about some factors influencing the size of the stroke volume.

Material

Sixteen cases with funnel chest of varying degree have been studied. There were 6 women and 10 men, ranging from 15 to 63 years of age. Values for age body weight and height are presented in table I All patients considered themselves to have a fairly normal degree of physical activity Prior to the investigation all underwent clinical examination including history examination of heart and lungs, X ray of the chest, ECG at rest and during exercise and routine blood and urine analysis. Apart from the chest deformity they showed no physical evidence of disease The smallest distance from the posterior part of the sternum to the anterior border of the vertebral body as estimated from the X-ray has been taken as a measure of the severity of the deformity Eight patients complained of some dyspnea and fatigue on exertion. Four patients

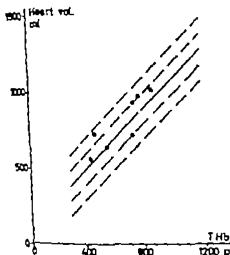


Fig. 1. Heart volume in relation to total amount of hemoglobin (THb)
— — normal regression lines obtained from determinations on 56 healthy subjects.
— — — — ± one and two standard errors of estimate (13)

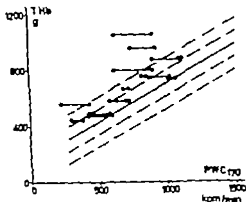


Fig. 2. Total amount of hemoglobin (THb) in relation to work intensity at pulse rate 170 (PWC_{170})
- PWC_{170} in the supine,
- PWC_{170} in the sitting position.
See also fig. 1

Radiological examination of the chest showed displacement to the left and a flattened appearance of the heart in 12 cases. In 4 cases no or only slight displacement of the heart was observed. The flattened shape was however evident also in these cases. The distance from the posterior surface of the body of the sternum to the anterior border of the vertebral body as measured from the X-ray varied between 3.5 and 9.3 cm (mean value = 6.0 cm). In 6 cases this distance was 5 cm or less (table 1).

The total amount of hemoglobin (THb) was for the male group 816 g (range 577—1,047) corresponding to 12.2 g per kg body weight and for the female group 534 g (range 445—663) corresponding to 9.5 g per kg body weight. These values do not show any significant difference from those found in a series of normal subjects (13).

The hemoglobin concentration was on an average normal and amounted for the males to 14.6 g per 100 ml blood (range 13.2—17.3) and for the females to 12.5 g (range 11.6—13.6).

The *blood volume* was 83.1 ml per kg body weight (range 67.1—97.8) for the male group and 75.8 ml per kg body weight (range 68.4—84.3) for the female group. These values do not deviate significantly from those found in normal subjects (15).

The *heart volume* in the prone position in relation to the THb was in 9 cases within two times the standard error of estimate (fig. 1). Only in case no 15 was the heart definitely large in relation to the THb. Due to the chest deformity the right heart border could not be outlined in 5 of the cases and no value for the heart volume was therefore obtained in these cases.

The *physical working capacity* at pulse rate 170 (PWC_{170}) was for the male group

Table I Some anthropometric data of 16 patients with funnel chest

Case no.	Sex	Age, years	Height, cm	Weight kg	B. S. A., m	Sterno-vertebral dist., cm	Heart volume, ml	Total hemoglobin g	Blood volume L	Work intensity kpm/min. at pulse rate 170 beats per min.		Pulse rate supine	Pulse rat standing	Vital capacity L	Total capacity L	Residual quotient %
										Sitting	Supine					
1	M	15	187	62.2	1.88	6.0	600	—	—	780	830	92	116	3.78	5.28	28
2	M	17	191	71.5	2.03	7.0	930	75	4.8	820	850	80	103	3.86	5 77	33
3	M	20	182	75.4	1.98	4 0	—	1 047	6.0	620	900	94	123	5.79	7.67	25
4	M	20	159	42.2	1 40	5.5	590	577	3.8	580	700	72	107	3.38	4 36	23
5	M	22	184	67.5	1.91	9.5	—	867	6.6	900	1 090	70	118	6.32	8.17	25
6	M	24	179	77 0	1.97	6 0	—	734	5.4	1 010	1 060	62	92	4.25	5 67	25
7	M	24	189	64.5	1.92	5.0	970	792	5.9	620	900	52	83	5.34	6.75	21
8	M	25	182	68.0	1.90	4.5	—	951	6.2	740	970	80	118	3.54	5.02	29
9	M	26	176	73.5	1.90	9.0	1 010	877	5 8	1 110	1 090	64	108	4 00	5.70	36
10	M	33	174	66.0	1.82	8.5	710	745	5 4	870	1 020	62	91	4.19	5.69	26
11	F	16	172	63.5	1 76	7.5	750	575	4.6	630	710	71	106	4.24	5.38	21
12	F	19	157	47 4	1 45	6.5	560	445	3.3	300	380	92	128	2.78	3 91	29
13	F	27	169	49.8	1.58	4 0	725	485	4 2	430	580	7*	114	3 49	5.60	38
14	F	34	167	55.8	1.63	5.5	640	560	4.5	220	430	80	98	3 67	4.98	26
15	F	54	168	58.5	1 67	4.5	890	473	4.0	430	600	73	102	2 76	3 91	29
16	F	63	171	63 0	1 75	3.5	—	663	5.0	720	680	65	91	—	—	—

Not in "steady state

guidance of the earlier determined physical working capacity (PWC_{170}) so that the final pulse rate on the highest work load in the sitting position was above 150 beats per minute in most cases.

Results

Physical findings An early systolic murmur of low frequency and not stronger than grade 2—3 was present over the pulmonic area in 6 cases. It was evaluated as a physiological murmur The second heart sound was split in 6 cases The splitting ranged from 0 04 to 0 08 seconds and varied in a normal way with respiration.

The *ECG* showed in 9 cases minor right intraventricular conduction disturbances. In one case (no 12) the ECG at rest showed inverted T waves in lead CR_{II} CR_3 and CR_4, which could be explained by the abnormal position of the heart Orthostatic ECG-changes with inverted or diphasic T waves were present in 6 cases (no 3 6 8 9 12 and 13) In these cases the ECG also showed during exercise changes of the same type as during the orthostatic test. In case no 13 these changes persisted a few minutes after exercise

The average *pulse rate* at rest in supine position was 73 8 beats per minute (range 52—94) and in standing position 106.1 beats per minute (range 83—128) At the *orthostatic test* the pulse rate increased more than 20 beats per minute and to a level above 100 in 11 of the cases (table I)

Table II. Data obtained in connection with right heart catheterization in 15 cases with funnel chest

Case no.	Position	Work load, kpm/min.	Pulse rate, beats/min.	Oxygen uptake, ml/min.	Mechanical efficiency, %	O_2-capacity, ml/100 ml	O_2-sat. % Br A.	O_2-sat. % P A.	AV-O_2-diff., ml/L	Card. output, L/min.	Stroke volume, ml	Pressures, mm Hg RV S	RV De	PA S	PA D	PA M	PCV M	Br A S	Br A D	Br A M
1	R	rest	89	282		16.9	98	77	36	7.8	87	23	3	20	12	13		103	57	73
	S	rest	120	356		17.8	97	68	53	6.7	56	27	—	22	11	—		92	60	—
	S	300	145	841	23	18.6	93	51	85	9.9	68	42	3	30	—	20		124	72	89
	S	600	180	1,388	26	18.6	97	56	117	11.8	64	43	6	29	13	20		115	55	73
	R	rest	94	302		16.0	97	76	33	8.6	91	25	5	20	6	14	10	103	[illegible]	69
	R	300	128	920	22	16.5	97	54	73	12.6	98	37	5	34	11	21	10	121	51	77
	R	600	133	1,468	24	17.8	96	45	88	16.8	110	44	6	39	7	23	11	157	62	89
2	R	rest	87	309		20.5	93	78	45	6.8	79	22	4	18	6	12	7	120	73	91
3	R	rest	74	288		22.3	99	84	33	8.2	111	23	3	21	8	13	8	125	70	92
	S	rest	111	366		23.4	98	70	69	5.3	48	23	1	16	7	11	—	—	—	85
	S	250	130	865	20	23.8	98	57	99	8.7	67	34	5	21	7	14		116	80	102
	S	500	159	1,319	23	23.9	90	42	138	9.6	60	42	6	27	8	17		131	80	97
	R	rest	73	287		22.0	98	80	40	7.1	98	22	6	18	7	13	8	133	70	91
	R	250	93	838	21	22.1	97	63	77	10.8	117	—	—	23	8	16	10	139	68	87
4	R	rest	105	311		19.1	99	83	32	9.8	93	25	1	18	7	13	7	120	72	91
5	R	rest	88	312		20.2	98	87	25	12.7	150	17	1	12	4	8		127	58	83
	S	rest	126	375		20.1	99	67	67	5.6	45	21	3	20	5	12		115	73	90
	S	400	134	1,205	20	21.2	93	44	119	10.1	63	31	2	20	13	17		139	70	92
	S	600	173	1,500	23	21.1	99	34	140	10.7	62	56	—	19	12	16		130	60	84
	R	rest	96	292		19.3	99	84	30	9.6	100	17	0	14	6	9	6	127	63	79
	R	400	128	1,111	22	19.5	97	60	74	13.0	117	33	1	26	8	16	8	—	—	—
	R	600	149	1,633	21	20.4	98	51	89	16.5	111	38	0	30	5	15		154	64	97
6	R	rest	60	318		18.6	95	76	40	8.0	133	22	5	20	9	14		129	77	93
	S	rest	75	455		19.4	96	66	59	7.4	98	21	2	20	11	14		131	80	100
	S	400	108	1,135	21	20.0	96	47	101	11.2	104	28	3	28	12	17		149	78	100
	S	800	154	1,986	22	20.8	96	36	125	15.9	103	52	3	31	14	21		173	82	107
	R	rest	76	300		18.4	96	78	36	8.4	111	25	4	19	10	14		127	70	92
	R	400	107	1,142	21	19.4	95	34	82	14.0	131	32	0	31	14	21		156	75	98
	R	800	139	1,639	27	19.7	96	48	97	17.0	122	32	2					174	77	109
7	R	rest	63	270		18.6	96	78	35	7.7	122	23	4	21	10	14		119	60	77
	S	rest	70	278		19.7	98	70	57	4.9	70			18	10	13		128	72	91
	S	250	104	775	23	20.7	96	53	91	6.5	82			20	10	14		149	80	99
	R	rest	53	230		18.5	97	77	38	6.0	113			18	8	12		118	57	73
	R	250	99	831	21	18.9	97	59	74	11.3	114			22	9	15		148	70	87
8	R	rest	72	287		20.4	99	80	40	7.1	99	23	3	23	8	12	8	118	63	81

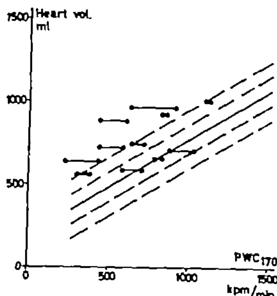


Fig 3 Heart volume in relation to work intensity at pulse rate 170 (PWC_{170}) Symbols as in fig 1 and 2.

805 kpm/min. (range 580—1 110) in sitting and 936 kpm/min (range 700—1 090) in supine position For the female group the values were 455 kpm/min (range 220—720) and 563 kpm/min. (range 380—710) respectively For the whole series the PWC_{170} was on an average lower in sitting than in supine position by 123 kpm/min corresponding to 15 4 per cent The difference is significant ($p < 0.001$) In relation to the THb the PWC_{170} was somewhat low also in the supine position for some of the cases (fig 2) But only in two cases the deviation exceeded two standard errors of estimate. Also in relation to the heart volume the PWC_{170} was low for several cases both in sitting and supine position (fig 3) In five cases the difference exceeded two standard errors of estimate both in sitting and supine position. In the group of cases where the PWC_{170} in sitting position was lower by 20 per cent or more than in supine position the sterno-vertebral distance was on an average shorter than in the other group The difference is probably significant ($p < 0.02$) The scatter is however large and the oldest patient (no 16) who had the shortest sterno-vertebral distance had approximately the same PWC_{170} in sitting as in supine position.

Lung volumes Compared to normal material (9) the vital capacity was reduced in several cases (table I) In some cases the total capacity was also somewhat low The residual quotient was somewhat increased in two cases (no 2 and 13) There was no correlation between the degree of orthostatic reaction and the reduction of the lung volumes.

Data obtained during heart catheterization

Cases no 2 4 8 and 13 were examined only in supine position at rest, except for no 13 who was examined during exercise also In case no 4 and 13 oxygen uptake and pulse frequency were considerably higher than what had been found during basal conditions prior to catheterization. However the other cases were relatively unchanged. Besides this the hemodynamic findings were completely normal in these cases (table II)

The remaining 11 cases have been examined at rest and during exercise both in sitting and supine position and the results reported below are confined to these cases. All investigations were carried out without complications.

Pulse rate at rest in the supine position during the first determination of cardiac output was 75.2 beats per minute (range 60—98) and during the second determination 74 7 beats per minute (range 53—94) After 6—7 minutes resting on the bicycle it was 96 0 beats per minute (range 70—126) corresponding to an increase in pulse rate of 20 8 beats per minute The pulse rate counted at rest in supine position before heart catheter

Table II (cont.)

Case no.	Position	Work load, kpm/min	Pulse rate beats/min	Oxygen uptake ml/min	Mechanical efficiency %	O_2-capacity ml/100 ml	O_2-sat. % Br A.	O_2-sat. % P A.	AV-O_2 diff. ml/L	Card. output, L/min	Stroke volume ml	Pressures mm Hg RV S	RV De	PA S	PA D	PA M	PCV M	Br A. S	Br A. D	Br A. M
16	R	rest	64	183		17.2	96	73	39	4.7	74	22	5	21	8	14	9	126	66	90
	S	rest	81	331		17 7	96	66	55	6 0	74	21	2	18	10	13		136	80	106
	S	200	103	782	16	18 6	98	40	106	7 4	72	37	2	29	13	20		157	78	109
	S	400	129	1 134	20	20.1	97	32	132	8 6	67	38	2	36	11	22		153	73	101
	R	rest	64	183		16.4	96	76	34	5.4	84	21	5	21	9	13		121	64	86
	R	200	93	679	19	17.3	96	48	84	8.1	87	44	12	43	18	29	19	160	72	109
	R	400	122	1,140	20	17 4	96	39	100	11 4	93	51	3	42	16	28		197	81	128

S = Sitting, R = Recumbent, Br A. = Brachial artery RV = Right ventricle, PA Pulmonary artery PCV = Pulmonary capillary venous, S = Systolic, De = End-diastolic, M = Mean.

lization was 73.6 beats per minute (range 52—94)

Pulse rate during exercise In all cases was higher at the same work load in sitting than in supine position. The mean difference as 18.2 beats per minut (range 1—37) and 18.4 beats per minute (range 7—29) on the first and second work loads respectively

Oxygen uptake at rest in the supine position during the first determination was 14.8 per cent (range + 2— + 33) higher than the predicted basal value (11) During the second determination at rest in the supine position t was 8.9 per cent (range — 7— + 23) and at rest sitting on the bicycle 40 1 per cent (range + 12— 82) higher than the predicted value.

Oxygen uptake during work There was no significant difference in oxygen uptake between work in the sitting and supine position. As the work loads were the same in both positions, it follows that the mechanical efficiency was the same which on the highest work load was an average of 22 7 per cent in sitting and 22.1 per cent in supine position

The oxygen saturation of the arterial blood varied within normal limits at rest as well as during work. The oxygen capacity of the blood increased significantly during work and in about the same degree as in normals (2)

The oxygen saturation of mixed venous blood at rest and during exercise as in normal subjects (2) in all cases was lower in sitting than in supine position. At rest it amounted to 69.0 per cent (range 66—73) in sitting and 78.1 per cent (range 74—84) in supine position. During the highest work load it decreased to 36 1 per cent (range 30—46) and 45 5 per cent (range 38—51) respectively The differences between sitting and supine posture are significant ($p < 0.001$) In fig 4 and 8 the oxygen saturation in mixed venous blood is shown in relation to pulse rate.

Table II (cont.)

Case no	Position	Work load, kpm/min.	Pulse rate, beats/min.	Oxygen uptake, ml/min.	Mechanical efficiency %	O_2-capacity ml/100 ml	O_2-sat. % Br A.		AV-O_2-diff.	Card. output, L/min.	Stroke volume, ml	Pressures mm Hg RV S		PA S			PCV M	Br A. S		
							Br A.	P A.				RV S	RV De	PA S	PA D	PA M	PCV M	Br A. S	Br A. D	Br A. M
9	R	rest	65	294		20.1	98	80	39	7.6	117	16	−1	11	4	7		119	65	83
	S	rest	91	345		20.6	96	72	51	6.7	74	19	−4	19	6	11		142	89	114
	S	400	119	1 178	20	21.7	97	50	103	11.4	96	29	2	23	15	17		147	83	103
	S	800	153	1,881	23	21.7	97	41	125	15.3	100	37	−3	25	18	21		176	88	119
	R	rest	66	270		19.5	98	78	43	6.3	95	17	3	15	5	9	4	111	62	80
	R	400	107	1 196	20	20.8	98	54	93	12.8	120	23	1	18	7	12	6	142	74	92
	R	800	146	1,879	23	21.3	95	47	104	18.1	124	37	−1	21	11	16		169	72	103
10	R	rest	75	2 0		20.5	96	78	37	7.3	98	22	2	17	4	10	5	123	63	82
	S	rest	94	282		20.8	98	73	52	5.4	58	26	4	18	6	10		154	85	100
	S	400	135	1 124	21	21.6	95	47	108	10.5	77	39	4	23	11	16		164	77	99
	S	800	1 0	1 727	25	21.9	95	34	131	13.2	78	45	5	27	11	17		140	65	94
	R	rest	77	235		20.1	97	78	41	5.8	75	21	2	16	5	9	3	127	70	89
	R	400	108	1,034	23	20.4	97	55	88	11.9	110	29	−1	17	4	10	4	148	60	86
	R	800	159	1 754	25	20.8	94	45	103	17.1	107	44	0	33	6	15		176	68	93
11	S	rest	99	302		17.1	98	69	51	6.0	60	19	0	18	10	12		114	68	86
	S	250	127	767	21	17.8	96	43	96	8.0	63	23	4	22	11	16		126	70	90
	S	500	169	1 186	24	18.0	97	30	111	10.7	63	37	6	25	12	19		123	74	82
	R	rest	83	218		15.6	99	77	37	6.0	72	19	5	17	8	12		108	62	76
	R	250	107	775	21	16.1	99	45	90	8.7	81	26	6	22	13	18		126	60	83
	R	500	140	1,238	23	16.6	97	38	100	12.3	68	37	3	[illegible]	13	18		160	67	93
12	R	rest	97	191		16.3	99	82	30	6.3	67	21	3	15	6	11	6	108	68	81
	S	rest	111	208		17.2	97	70	49	4.3	38	21	2	20	7	11		100	70	82
	S	300	162	732	25	18.0	96	38	108	6.8	42	31	0	24	7	13		100	66	81
	R	rest	91	203		15.5	99	81	32	6.4	71	21		20	7	13	7	104	67	83
	R	300	141	851	21	16.3	97	46	86	9.9	70	35	2	26	10	16	9	153	7	101
13	R	rest	99	241		16.3	100	84	32	7.7	77	1	2	20	9	13	6	113	85	96
	R	200	126	753	18	16.3	100	59	68	10.7	85	32		31	11	21	8	120	79	98
	R	400	159	970	24	17.1	100	49	89	10.9	68	28	4	27	12	20		116	80	96
14	R	rest	67	253		16.5	100	78	38	6.6	99	22	3	20	5	11		117	61	82
	S	rest	81	—		17.1	99	68	55		—	30	0	22	9	—		—		—
	S	112	112	603	14	18.1	96	36	74	8.1	73	40	0	23	11	16		160	88	111
	S	200	142	749	18	18.6	96	46	96	7.8	55	47	1	24	10	15		157	89	109
	R	rest	57	234		15.8	97	74	39	6.1	107	23	4	17	6	11		125	60	83
	R	112	90	596	14	16.5	95	58	63	9.5	106	30	3	27	7	16		149	76	97
	R	200	115	888	14	16.8	98	51	80	11.1	94	36	3	30	9	17		151	73	97

Table II (cont.)

Case no.	Position	Work load, kpm/min.	Pulse rate, beats/min.	Oxygen uptake, ml/min.	Mechanical efficiency %	O_2-capacity ml/100 ml	O_2-sat. % Br A.	O_2-sat. % P A.	AV-O_2-d (L, ml L	Card. output, L/min.	Stroke volume, ml	Pressures mm Hg RV S	RV De	PA S	PA D	PA M	PCV M	Br A. S	Br A. D	Br A. M
16	R	rest	64	185		17.2	96	73	39	4.7	74	22	3	21	8	14	9	126	68	90
	S	rest	81	331		17 7	96	66	55	6 0	74	21	2	18	10	13		136	80	106
	S	200	103	782	16	18 6	96	40	106	7 4	72	37	2	29	13	20		157	78	109
	S	400	129	1,134	20	20.1	97	52	132	8 6	67	38	2	36	11	22		153	73	101
	R	rest	64	183		16.4	96	76	34	5 4	84	21	5	21	9	13		121	64	86
	R	200	93	679	19	17.3	96	48	84	8.1	87	44	12	43	18	29	19	160	72	109
	R	400	122	1,140	28	17 4	98	39	100	11 4	93	51	3	42	16	28		197	81	128

S = Sitting, R = Recumbent, Br A. = Brachial artery RV = Right ventricle, PA = Pulmonary artery PCV = Pulmonary capillary venous, S = Systolic, De = End-diastolic, M = Mean.

bation was 73.8 beats per minute (range 52—94)

Pulse rate during exercise in all cases was higher at the same work load in sitting than in supine position. The mean difference was 18.2 beats per minute (range 1—37) and 18 4 beats per minute (range 7—29) on the first and second work loads respectively

Oxygen uptake at rest in the supine position during the first determination was 14.8 per cent (range + 2—+ 33) higher than the predicted basal value (11) During the second determination at rest in the supine position it was 8.9 per cent (range — 7—— 23) and at rest sitting on the bicycle 40 1 per cent (range + 12—+ 82) higher than the predicted value.

Oxygen uptake during work. There was no significant difference in oxygen uptake between work in the sitting and supine position As the work loads were the same in both positions, it follows that the mechanical efficiency was the same, which on the highest work load was an average of 22.7 per cent in sitting and 22.1 per cent in supine position.

The oxygen saturation of the arterial blood varied within normal limits at rest as well as during work. The oxygen capacity of the blood increased significantly during work and in about the same degree as in normals (2)

The oxygen saturation of mixed venous blood at rest and during exercise as in normal subjects (2) in all cases was lower in sitting than in supine position. At rest it amounted to 69.0 per cent (range 66—73) in sitting and 78.1 per cent (range 74—84) in supine position. During the highest work load it decreased to 36.1 per cent (range 30—46) and 45.5 per cent (range 38—51) respectively The differences between sitting and supine posture are significant ($p < 0.001$) In fig 4 and 8 the oxygen saturation in mixed venous blood is shown in relation to pulse rate.

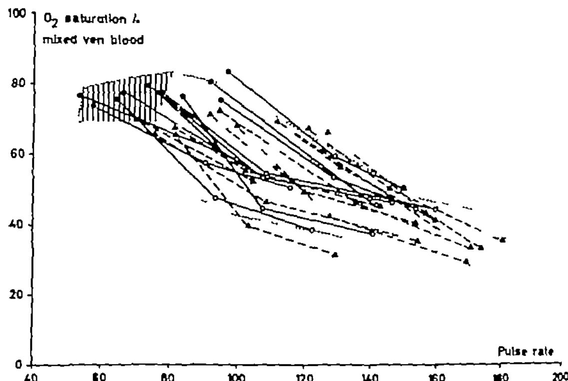


Fig 4 O_2-saturation of the blood in pulmonary artery in relation to pulse rate at rest and during exercise.

— values at rest — values during work. •— — = values for each case in the supine position.

▲— = triangles connected by dashed lines refer to th sitting position.
△—

The area within the dotted line indicates the normal variation, obtained from determinations on 27 healthy subjects in the supine position (2, 16) The shaded area represents the variations at rest.

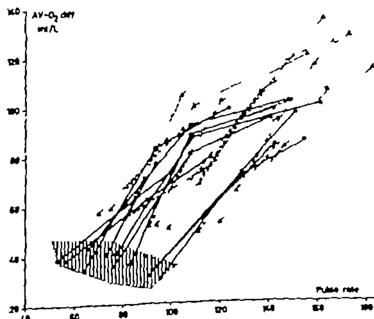


Fig 5. Arterio-venous oxygen difference in relation to pulse rate at rest and during work. Symbols as in fig 4

The arterio-venous oxygen difference at rest was 56.2 ml/l (range 49—69) in sitting and 56.7 ml/l (range 50—43) in supine position. It increased on the highest work load to 121.9 ml/l (range 96—140) and 96.3 ml/l (range 80—104) respectively. In relation to pulse rate it was within normal limits in all cases in the supine position (fig 5 and 8). As in normal subjects (2) it was in all cases higher in sitting than in supine position. The differences are significant ($p < 0.001$)

The cardiac output at rest was 5 82 l/min (range 4.3—7 4) in sitting and 6 97 l/min (range 5 4—9.6) in supine position. On the highest work load this increased up to 11 75 l/min (range 7.8—15.9) and 13.02 l/min (range 11 1—18.1) respectively. The differences between sitting and supine position (fig 6) are significant ($p < 0.001$ during exercise) and of the same order as in normal subjects (2). Regression equations for cardiac output in relation to oxygen uptake at rest and during exercise in sitting and supine position showed no significant deviation when compared with those obtained from a larger series of normal subjects (2 16). Consequently there is no significant difference from the normal subjects concerning the arterio-venous oxygen difference in relation to the oxygen uptake either

The stroke volume at rest during the first determination in supine position was 103.8 ml (range 6 —133) and during the second was 94.5 ml (range 71—113). The difference is not significant and the coefficient of variation amounts to 10.6 per cent, which is close to the error of the method (19). In the supine position the stroke volume in relation to the blood volume was within normal limits at rest and during work (fig. 7)

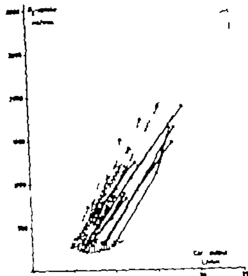


Fig 6. Oxygen uptake in relation to cardiac output at rest and during exercise. Symbols as in fig 4.

On changing from a supine to a sitting position the stroke volume decreased in all patients, except in the oldest one (no 16) where it was unchanged. The average decrease of the stroke volume which amounted to 40 3 per cent, was of the same order as in normal subjects (2)

In supine position the stroke volume increased from rest to work on an average by 13.2 per cent. This increase is significant ($p < 0.01$). However during continued exercise with increasing intensity the stroke volume remained constant (fig 8). The coefficient of variation calculated from the differences in stroke volume between the two work loads amounted to 5.5 per cent, which is within the error of the method (19). The stroke volume during work in the supine position was on the average 2.01 per cent of the blood volume.

In a sitting position on transition from rest to exercise, the stroke volume also

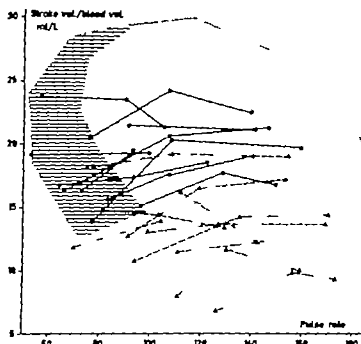


Fig 7 Stroke volume divided by blood volume in relation to pulse rate at rest and during exercise. Symbols as in fig 4.

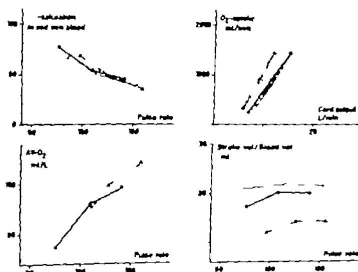


Fig. 8. Mean values of some hemodynamic relationships at rest and during exercise in 11 cases with funnel chest (heavy lines) Symbols as in fig 4 For comparison mean values obtained from 8 healthy male subjects are plotted (2) The normal values are represented by small dots connected by thin straight lines for supine and the dashed lines for sitting position.

showed significant increase ($p < 0.01$) This was on an average 18 5 per cent and significantly lower ($p < 0.001$) than in normal subjects where it was 51 per cent (2) With increasing work intensity the stroke volume on an average remained constant and was thus also with a heavier work load considerably lower in sitting than in supine position (fig 8) It was on an average 31 0 per cent lower in the sitting position This difference is significantly larger ($p < 0.01$) than in normal subjects (2)

The mean value for the stroke volume during exercise in supine position in relation to heart volume was in several

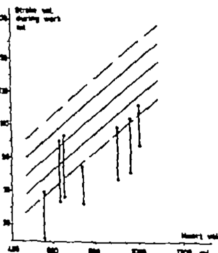


Fig. 9 The relationship between the stroke volume (mean of the values obtained during work) and the heart volume. • = supine position. ○ = sitting position. — = regression line ± one standard error of estimate. — — — = ± two standard errors of estimate obtained from a series of normal subjects (16). Regression equation: y = 27.0 + 0.1x, r = 0.86, S. D. = 10.9, n = 18

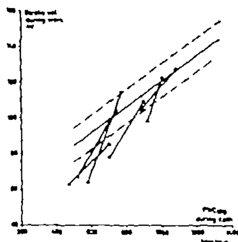


Fig. 11 Stroke volume (mean of values obtained during work) in relation to work intensity at pulse rate 170 beats per minute (PWC_{170}) during heart catheterization. • = supine position. Δ = sitting position. — = normal regression line for supine position. — — — = ± one standard error of estimate.

The normal values are obtained from determinations on 27 healthy subjects (2, 16).

Regression equation: y = 49.1 + 0.072x, r = 0.88, S. D. = 9.5.

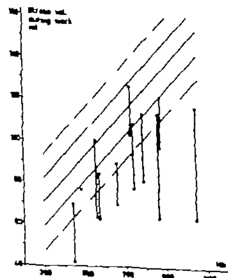


Fig. 10 Stroke volume (mean of values obtained during work) in relation to total amount of hemoglobin (THb). Symbols as in fig. 9.

Regression equation: y = 37.2 + 0.11x, r = 0.84, S. D. = 11.5, n = 18. (16)

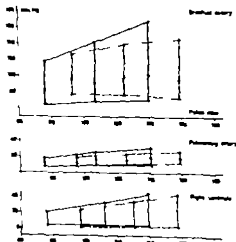


Fig. 12 Mean values of pressures from brachial artery, pulmonary artery and right ventricle at rest and during exercise.

Symbols as in fig. 4

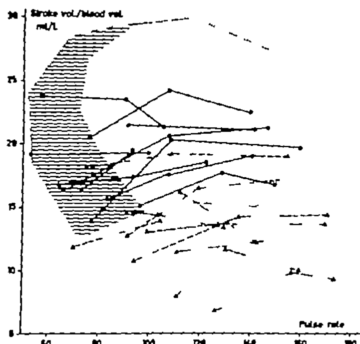


Fig. 7 Stroke volume divided by blood volume in relation to pulse rate at rest and during exercise Symbols as in fig 4

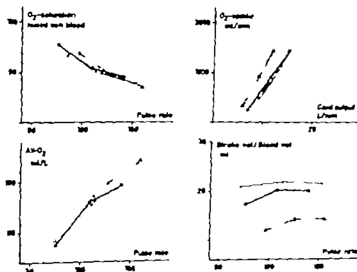


Fig 8. Mean values of some hemodynamic relationships at rest and during exercise in 11 cases with funnel chest (heavy lines) Symbols as in fig 4 For comparison mean values obtained from 8 healthy male subjects are plotted (2) The normal values are represented by small dots connected by thin straight lines for supine and thi dashed lines for sitting position.

showed significant increase ($p < 0.01$) This was on an average 18.5 per cent and significantly lower ($p < 0.001$) than in normal subjects, where it was 51 per cent (2) With increasing work intensity the stroke volume on an average remained constant and was thus also with a heavier work load considerably lower in sitting than in supine position (fig 8) It was on an average 31.0 per cent lower in the sitting position This difference is significantly larger ($p < 0.01$) than in normal subjects (2)

The mean value for the stroke volume during exercise in supine position in relation to heart volume was in several

were present. *The subjective symptoms*, found in 11 cases were vague, predominantly being fatigue and dyspnea on exertion. The complaints were similar to those described in patients with so-called vasoregulatory asthenia (13) and might be explained by the reduced physical working capacity in an upright position.

The objective heart findings consisted of displacement and altered shape of the heart, systolic murmurs over the pulmonic area, an occasionally marked splitting of the second sound and minor deviations in the ECG-pattern. The systolic murmurs, being of low frequency and not stronger than grade 2—3 were evaluated as being of physiological origin. Systolic murmurs of this type are common in cases with funnel chest (29, 6, 30, 7 23) and probably can be explained by favourable sound transmission due to the short distance between the pulmonary artery and the anterior chest wall. Diastolic murmurs, as well as more intense and high frequency systolic murmurs, can not be explained by the chest deformity and should give rise to suspicion of associated heart disease. Fabricius et al (7) found 4 patients with associated heart disease among 26 cases of funnel chest. Accentuated and split second heart sound has been reported also in other series (6, 30, 22) Accentuation of the second sound could, as well as the systolic murmur be explained by favourable sound transmission.

The ECG in 10 cases showed a small right intra ventricular conduction disturbance (rSr pattern in lead V_1) which seems to be a common finding in funnel chest (39 28 22) Martins de Oliveira et al. (28) are of the opinion that this is no sign of conduction disturbance, but is related to a rotation and dislocation of the heart.

The relatively common occurrence of a systolic murmur split second sound and ECG with rSr'-pattern in lead V could give rise to suspicion of an atrial septal defect. In this group there were no signs of left to right shunt from the heart catheterization. A very small shunt, however can not be excluded by this method.

Respiratory function. The lung volumes were somewhat small in several cases, but in none did respiratory function appear to be impeded, neither at rest nor during work of submaximal intensity as judged by the normal values for arterial oxygen saturation. Neither did clinical observation during exercise tests reveal signs of respiratory distress.

Heart catheterization involves procedures that may change the functions to be measured. Judging by the values at rest for pulse rate and oxygen uptake, most patients reported here were in a fairly basal condition.

During exercise, however some cases showed a higher pulse rate in relation to the work load, than before heart catheterization. Before the second work test started restitution of pulse, oxygen uptake and cardiac output had occurred.

Cardiac function

Disturbances in cardiac function have earlier been reported (33 26, 39) Thus Wachtel et al. (39) express the opinion that in moderate and severe cases the deformity may result in profound disturbances of cardiorespiratory physiology" It is, however difficult to judge the validity of these findings, as the series have been small and methods and data often incompletely reported. In some cases associated heart disease, not related to the deformity may have been present. In a recent work Bergh and Berglund (1)

cases somewhat low but only in two cases (no 11 and 13) the deviation exceeded two times the standard error of estimate for normals (fig 9) In relation to the THb it was also somewhat low in some cases but only in case no 3 was the deviation considerable (fig 10) Six cases had a final pulse rate on the highest work load in supine position above 140 beats per min. In these cases the expected value of the PWC_{170} during heart catheterization has been calculated both for supine and sitting posture. The decrease in stroke volume in sitting position was more marked than the decrease in PWC_{170} (fig 11)

Intracardiac and intravascular pressures The mean values are presented in fig 12 and the individual values in table II The pressures in the brachial artery pulmonary artery and right ventricle, both in sitting and supine position at rest and during work were within normal limits (2) As will be seen from fig 12 the pulse amplitude in the brachial and pulmonary arteries at rest and during exercise was on the average smaller in sitting than in supine position. This is probably a consequence of the smaller stroke volume in this posture. The pulmonary arterial wedge pressure was also normal in all cases where it was recorded In the oldest patient (no 16) it increased from 9 to 19 mm Hg on 200 kpm/min With regard to age this increase cannot be considered as pathological (8) The end-diastolic pressure of the right ventricle was within normal limits in all cases. It showed in the sitting position a tendency to increase during exercise. However this increase was not significant In supine position it decreased slightly ($p < 0.02$) The flow resistances in the pulmonary and systemic circulations were within normal limits in all cases where they could be calculated.

Discussion

This series includes both female and male subjects. Apart from smaller dimensions of the cardiovascular system and of other body parameters, the performance of the female group did not deviate from that of the male group with regard to the results of this study Both groups are therefore considered together in the discussion.

The most prominent circulatory finding in the present material of patients with funnel chest is a pulse rate, that during exercise with a given load, is higher in the sitting than in the supine position, which for the purpose of discussion, is called the orthostatic pulse reaction during work. This results in a significant decrease of the physical working capacity at a pulse rate of 170 beats per minute (PWC_{170}) in the sitting position. In a series of normal subjects (2) and in a group of patients hospitalized for various diseases (3) no systematic difference has been found between the PWC_{170} in sitting and supine position.

Judging by the history the physical activity could be considered of ordinary degree in most cases, and two patients, (no 8 and 9) had earlier even taken part in athletics. Only two patients (no 7 and 13) lead a more sedentary life. It is not known if physical inactivity may cause an orthostatic pulse reaction.

A marked orthostatic pulse reaction during exercise has been found in patients with pulmonary sarcoidosis and it has been suggested that the mechanism could be a lower venous tone in these cases (38) In this series of patients with funnel chest there were no pulmonary changes to be seen on plain X ray

In this material certain subjective and objective cardiopulmonary symptoms

were present. *The subjective symptoms* found in 11 cases were vague, predominantly being fatigue and dyspnea on exertion. The complaints were similar to those described in patients with so-called vasoregulatory asthenia (13) and might be explained by the reduced physical working capacity in an upright position.

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found improvement of cardiac function after operative treatment in a few patients.

In the series presented in this report, some deviations from the normal pattern were found. However the hemodynamic conditions were normal in the supine position and there was no evidence of heart disease in any of the cases. Some investigators (27 39 25 20) have suggested that a marked funnel chest may restrict the diastolic expansion of the ventricles and produce a picture similar to constrictive pericarditis. In the present material of patients with funnel chest, a normal stroke volume was maintained at rest and during exercise in supine position with normal filling pressures. This is evidence against restriction for the diastolic expansion of the ventricles either from pressure exerted by the thoracic depression or from decreased myocardial distensibility From the roentgenological examinations it also appeared that in most cases it is the right atrium and not the ventricles that are situated behind the depressed sternum Although the heart is thus to be considered as normal the orthostatic pulse reaction during exercise is an abnormal finding

The influence of body position

In young healthy males cardiac output decreases approximately by 2 l/min on changing from a supine to sitting position. At the same time the pulse rate increases and the stroke volume decreases by an average of about 40 per cent (2) This decrease of the stroke volume is probably caused by a shift of blood from the central circulation to the lower part of the body causing an impaired filling of the ventricles On transition from rest to exercise in a sitting position a redistribution of blood in a central direction occurs causing an increase of the stroke volume to a level slightly below that in the supine position (2) The venous pump of the leg muscles, the sympathetic tone adjusting the capacity of the venous system and the abdominothoracic pumping action (34) seem to be factors of importance for this redistribution of blood in central direction.

In patients with funnel chest the stroke volume decreased in a normal way on changing the posture from supine to sitting However on transition from rest to exercise, while sitting the stroke volume increased only slightly and was thus considerably lower than in a supine position even during exercise. Evidently these patients can not compensate in a normal way for gravitational influence on the distribution of the blood volume. This is only apparent during exercise.

Inadequate adjustment of the capacity of the venous system with changes of posture could explain the orthostatic reaction. It is more probable, however that the orthostatic reaction is related to their obvious thoracic deformity which diminishes the volume of the lower part of the thoracic cavity

To investigate whether the depressed sternum and the displacement of the heart could cause some degree of stenosis within the caval veins angiocardiography with injection of contrast medium into the inferior vena cava was performed in three cases (no 7 8 and 15) In none of these cases could any anatomical changes within the caval veins be observed.

In most cases the right atrium was situated behind the depression In collaboration with the radiological department at the Thoracic Clinic, angiocardiography with contrast injection into the inferior vena cava was performed at rest both in sitting and supine position,

two cases. The right atrium was ...ated immediately behind the posterior ...face of the thoracic depression. Es...cially in a sitting position, the right ...ium appeared to be small and revealed ...visible impression from the thoracic ...formity. As pointed out by Sjöstrand ...7) the amount of blood available in ...e venous reservoir in front of the left ...ntricle is an important factor for the ...aintenance of the stroke volume. Such ...venous reservoir is especially important ... that part of the vascular system, as the ...roke volume of the left ventricle is of ...mediate importance for the regulation ...f the systemic arterial pressure and ...eart rate. As for the right side of the ...eart, small volume of the right atrium ...an be expected to influence the filling ...f the right ventricle proportionally to ...he importance of atrial systole for ventric...ular filling. With increasing pulse rate, ...s during exercise, the diastole shortens and atrial systole will contribute to ventricular filling in a progressively larger amount. A mechanism of this kind can be expected to have greater influence in the upright position, presuming that the compressive effect, in this position, on the right atrium by the depressed sternum is more pronounced.

The respiratory pressure variations are of great importance for venous inflow to the thorax (4). The diminished space in the lower part of the thorax in cases with funnel chest may decrease the efficiency of the abdominothoracic pumping mechanism, which is important for ventricular filling. Lester (24) and Howard (20) point out that in children with funnel chest, the sternum may show paradoxical respiratory movements, which later in life are replaced by orthodox movements of small amplitude. As venous inflow to the thorax is accomplished by very small pressure differences, even a small reduction of the pressure gradient between the abdomen and the thorax may be important.

Although the present investigation does not allow any conclusions about the mechanism for the orthostatic pulse reaction during exercise in cases with funnel chest, it seems possible that the explanation may by found in a less efficient abdominothoracic pump or a diminished capacity of the right atrium.

The relationship between the stroke volume during exercise and the PWC_{170}

In normal subjects, the stroke volume during exercise is about 12 per cent lower in a sitting than in a supine position (2). The lower stroke volume in sitting position is compensated by a higher arteriovenous oxygen difference, so that the oxygen transport per pulse beat is the same in both positions. In the cases with funnel chest, the stroke volume is considerably smaller in sitting position, but peripheral oxygen utilization correlated to absolute work performed is approximately the same as in normal subjects. The considerably smaller stroke volume in sitting position is not fully compensated by an increase of the arterio-venous oxygen difference. Consequently the oxygen transport per pulse beat is lower in sitting position and results in a lower physical working capacity. The close relationship between the changes in stroke volume and PWC_{170} is shown in fig. 11.

Summary

1. A group of patients with funnel chest has been investigated and the results have been compared with earlier published data from a series of normal subjects.

2 The physical working capacity was significantly lower in sitting than in supine position by an average of 15 4 per cent. It did not appear to be limited by respiratory but by circulatory factors

3 Fifteen patients were investigated with the aid of right heart catheterization. Eleven of these cases were studied at rest and during exercise, both in sitting and supine position Although certain subjective and objective cardio-pulmonary symptoms were present, there was no evidence of any heart disease The hemodynamic findings were normal in the supine position

4 As in normal subjects the stroke volume at rest was 40 per cent lower in sitting than in supine position. On transition from rest to exercise, in sitting position, it increased significantly less than in normal subjects The stroke volume during exercise was 31 per cent lower in the sitting than in the supine position compared to an average of 12 per cent for normal subjects.

5 The arterio-venous oxygen difference varied normally in relation to oxygen uptake at rest and during work in both sitting and supine position The considerably lower stroke volume during exercise in a sitting position therefore resulted in a higher pulse rate at a given oxygen uptake and explained the lower physical working capacity

6 In this series there is no support for an opinion that the deformity should directly restrict the diastolic expansion of the ventricles, as normal stroke volumes are maintained with normal filling pressures in supine position.

7 The considerably smaller stroke volume during exercise in sitting than during exercise in supine position, is probably explained by impaired ventricular filling This investigation does not give any explanation for the mechanism of the impaired ventricular filling It is suggested however that the deformity may interfere with the efficiency of the abdominothoracic pumping mechanism. During exercise at higher pulse rates, a low capacity of the right atrium may also be of importance.

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Protein Pattern of Cerebrospinal Fluid in Meningitis and Meningo-encephalitis

By

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Paper electrophoretic studies of the cerebrospinal fluid in infectious diseases of the central nervous system (1, 2, 3) have revealed some qualitative changes of the proteins, the changes varying with the course of the disease. Any change in a single protein fraction must, however, be considerable before it can produce a significant abnormality of the pertinent protein group in paper electrophoresis or be demonstrated by salting out methods or by colloidal reactions. In contrast, immuno-electrophoresis utilizes not only the electrophoretic mobility of the proteins but also their immunologic specificity which increases the sensitivity of the method and thereby enables a more refined analysis of a particular protein fraction.

This paper is concerned with an immuno-electrophoretic investigation of the cerebrospinal fluid in meningitis and meningo-encephalitis. In nearly all of the cases cerebrospinal fluid was obtained within one week of the onset of the disease and before treatment had been started. In several cases further samples were collected in the later course of the disease, usually until the cerebrospinal fluid pattern had become normal.

Material and methods

The material consisted of the following cases, mainly from the Department of Infectious Diseases, University Hospital, Lund.

Nineteen patients, aged 6 to 54 years, with a firm clinical and serological diagnosis of mumps meningo-encephalitis.

Seventeen patients, aged 6 to 56 years, with another type of virus meningo-encephalitis. One of these patients had a serologically verified Russian-spring-summer encephalitis. In the other sixteen the type of the infection was unknown. No polio, echo, or C-virus could be demonstrated in the cerebrospinal fluid, blood or faeces in this last mentioned group. The number of the white cells in the cerebrospinal fluid was increased in all seventeen cases.

Four patients, aged 16 to 46 years, with clinical symptoms of meningo-encephalitis

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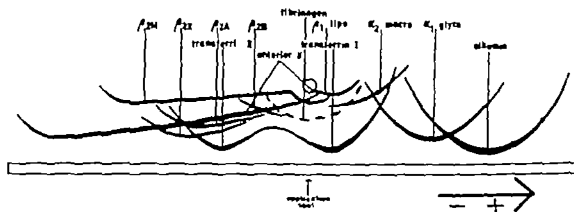


Fig 1 Model showing cerebrospinal fluid fractions studied. Some other fractions are also given for reference. Fibrinogen demonstrated with the aid of anti-fibrinogen serum and the other fractions with anti-human serum applied in the groove parallel to the electric field.

without co-existing signs of meningeal inflammation, *e g* encephalitis" Thus there was no increase in the number of cells or the total protein amount in the cerebrospinal fluid and the sugar and chlorides were also normal All four had neck-stiffness, headache and elevated body temperature in the acute stage and all showed electro-encephalographic abnormalities.

Five patients, aged 17 to 76 years, had bacterial meningitis (meningococcal in three, pneumococcal in one, and tuberculous in one)

The control group consisted of thirty patients, aged 12 to 66 years, who had sought advice for slight symptoms such as headache, dizziness and fatigue. Neurologic examination had revealed no abnormalities in any of them, and none had any severe mental symptoms.

Cerebrospinal fluid was obtained from all of the patients by lumbar puncture No specimens tinged with blood were accepted. In order to avoid any admixture of serum, the first few drops of cerebrospinal fluid were discarded. The fluid was centrifuged for 20 minutes immediately after collection and the cell-free supernatant was removed. The samples were concentrated by the method of Mies (4) by dialysis in collodion bags (Membranfiltergesellschaft, Göttingen, Germany) under negative pressure The concentration of the total proteins in the concentrate thus obtained was assessed spectrophotometrically by a modification of the technique described by Waddell (5) A standardized amount of the cerebrospinal fluid proteins was used throughout for immuno-electrophoresis. Immuno-electrophoresis (at pH 8.2 and ionic strength of 0 05) was performed by Heremans (6) modification of Scheidegger's (7) micromethod, which is based on the method of immuno-electrophoresis originally described by Grabar and Williams (8) Purified agar (Reinagar) and anti-human and anti-fibrinogen sera from rabbits were supplied by Behringwerke, Marburg an der Lahn, Germany The preparations were coloured with amido black 10 B (for proteins) and Sudan black (for lipoproteins) Details of the technique have been published elsewhere (9) In order to avoid errors due to antigen-antibody ratios deviating from the ideal for precipitation, tests were also carried out simultaneously with control sera and dilution series of the antigen and antibody respectively Only results obtained under ideal experimental conditions were accepted.

The immuno-electrophoretic protein pattern of the cerebrospinal *fluid* normally shows, on the average, 14 fractions (range 11 to 17) In neurologic diseases, *however* it shows a number of additional fractions, usually large-molecular blood constituents never or rarely seen in controls and then in only low concentration (9 10, 11 12)

Of these large-molecular proteins, the α_2-macroglobulin may occur *in the cerebrospinal* fluid in normal subjects. But in none of our controls did we find a marked α_2-macroglobulin precipitate of the type seen on immuno-electrophoresis of serum and indicating a substantially increased concentration of this fraction Nor have we ever observed

Table I. Survey of material, total protein values, and paper electrophoretic and immuno-electrophoretic findings in cerebrospinal fluid

Clinical groups	No. of cases	Total protein ≥ 60 mg/100 ml	P per electrophoresis %: β ≥ 20	P per electrophoresis %: γ ≤ 12	Immuno-electrophoresis: Normal pattern	Marked $\alpha_{2\gamma}$	β lipo	β_{1A}	β_{2M}	γ_{1M}	β_{1C}	Transferrin I only	Marked anterior γ	Fibrinogen
Control group	30	0	7	6	30	0	0	0	0	0	0	0	0	0
Mumps meningo-encephalitis	19	1	0	7	2	8	8	10	1	7	0	1	8	8
Other virus meningo-encephalitis	17	8	0	2	3	5	12	6	3	7	0	0	5	11
Encephalitis	4	0	0	2	0	0	2	0	0	0	2	0	0	4
Bacterial meningitis	5	5	0	3	0	5	3	3	0	3	1	1	3	4

β_1-lipoprotein, β_{1A}, β_{2M}, β_{1C} and β_2-macroglobulin, or fibrinogen in the cerebrospinal fluid in controls studied by the technique used in the present investigation.

Transferrin, the iron-binding protein, normally shows two precipitation arcs in the cerebrospinal fluid but only one, the anterior one, in serum. In neurologic disease, however, sometimes only the anodic transferrin arc can be obtained.

In the immuno-electropherograms of our thirty controls the large molecular part of the γ-globulin, the anodic part of the line, was either only faint or absent. In the patients with neurologic disorders, however, the concentration of the γ-globulin in this part of the line was often increased.

In the present investigation the cerebrospinal fluid was studied for the presence of γ-macroglobulin and transferrin of the type demonstrable in serum, β_1-lipoprotein, β_{1A}, β_{2M}, β_{1C} and β_2-macroglobulin as well as fibrinogen, and marked precipitation in the anodic part of the γ-globulin line containing the large-molecular part. Since no such findings were made in the control group, they are referred to below as "pathologic findings". The occurrence of these findings is given graphically in fig. 1, which also includes some normally occurring fractions for reference.

Results

The findings made in the controls and in the various infectious groups are summarized in table I. The numbers of white cells found in the cerebrospinal fluid are not included because no or only a few cells were found in the controls and because all of the patients with infectious disorders, except the four with meningo-encephalitis without meningeal inflammation, had an increased number of cells. It is clear from the table that the total proteins exceeded 60 mg/100 ml in only one patient with mumps meningo-encephalitis, but in as many as half of those with another type of virus meningo-encephalitis and in all of those with bacterial meningitis. The amounts of β- and γ-globulins as assessed by paper electrophoresis, and measured as percentage of the total proteins, were relatively large in one fifth of the controls in this series. The γ-globulin fraction, which might represent 17—18 per cent of the total proteins in the controls, was

Fig 1 Model showing cerebrospinal fluid fractions studied. Some other fractions are also given for reference Fibrinogen demonstrated with the aid of anti-fibrinogen serum and the other fractions with anti-human serum applied in the groove parallel to the electric field.

without co-existing signs of meningeal inflammation, e g encephalitis" Thus there was no increase in the number of cells or the total protein amount in the cerebrospinal fluid and the sugar and chlorides were also normal All four had neck-stiffness, headache and elevated body temperature in the acute stage and all showed electro-encephalographic abnormalities.

Five patients, aged 17 to 76 years, had bacterial meningitis (meningococcal in three, pneumococcal in one, and tuberculous in one)

The control group consisted of thirty patients, aged 12 to 66 years, who had sought advice for slight symptoms such as headache, dizziness and fatigue. Neurologic examination had revealed no abnormalities in any of them, and none had any severe mental symptoms.

Cerebrospinal fluid was obtained from all of the patients by lumbar puncture No specimens tinged with blood were accepted In order to avoid any admixture of serum the first few drops of cerebrospinal fluid were discarded The fluid was centrifuged for 20 minutes immediately after collection and the cell-free supernatant was removed. The samples were concentrated by the method of Miles (4) by dialysis in collodion bags (Membranfiltergesellschaft, Göttingen, Germany) under negative pressure. The concentration of the total proteins in the concentrate thus obtained was assessed spectrophotometrically by a modification of the technique described by Waddell (5) A standardized amount of the cerebrospinal fluid proteins was used throughout for immuno-electrophoresis. Immuno-electrophoresis (at pH 8.2 and ionic strength of 0 05) was performed by Heremans (6) modification of Scheidegger's (7) micromethod, which is based on the method of immuno-electrophoresis originally described by Grabar and Williams (8) Purified agar (Remagar) and anti-human and anti-fibrinogen sera from rabbits were supplied by Behringwerke, Marburg an der Lahn, Germany The preparations were coloured with amido black 10 B (for proteins) and Sudan black (for lipoproteins) Details of the technique have been published elsewhere (9). In order to avoid errors due to antigen-antibody ratios deviating from the ideal for precipitation, tests were also carried out simultaneously with control sera and dilution series of the antigen and antibody respectively Only results obtained under ideal experimental conditions were accepted.

The immuno-electrophoretic protein pattern of the cerebrospinal fluid normally shows, on the average 14 fractions (range 11 to 17) In neurologic diseases, however it shows a number of additional fractions, usually large-molecular blood constituents never or rarely seen in controls and then in only low concentration (9 10 11 12)

Of these large molecular proteins, the α macroglobulin may occur in the cerebrospinal fluid in normal subjects. But in none of our controls did we find a marked α_2-macroglobulin precipitate of the type seen on immuno-electrophoresis of serum and indicating a substantially increased concentration of this fraction Nor have we ever observed

Table II. Number of cells, total proteins and number of pathologic immuno-electrophoretic findings in cerebrospinal fluid found on repeated examination of 6 patients with mumps meningo-encephalitis

Sex	Age (years)	Day of disease	Cells per mm	Total proteins mg/100 ml	No. of pathologic findings
M	24	2	400	115	3
		91	2	47	0
F	44	1	98	31	1
		16	22.5	37	0
F	17	1	224	52	2
		63	2.3	29	0
M	7	3	372	35	3
		22	35	35	2
M	6	1	161	42	3
		12	9.4	30	1
F	8	31	171	112	7
		44	134	112	6
		56	24	58	3
		74	3.6	58	5
		130	3	36	2

six cases of mumps meningo-encephalitis followed up in this way are given in table II.

It is clear from the table that the number of pathologic findings tended to decrease with the recovery of the patient. This tendency was, however, not without exceptions, pathologic fractions sometimes persisting long after the total protein content and even the number of cells had become normal. Thus, in one case in which the patient (F 8) had recovered and the number of cells and the total protein content had become normal within 10 weeks of onset of the disease, 2 pathologic findings, namely the presence of β_{2M}-globulin and a marked anodic part of the γ-arc, persisted for at least a further 2 months.

Table III. Number of cells, total proteins, and number of pathologic immuno-electrophoretic findings in cerebrospinal fluid found on repeated examination of 8 patients with virus meningo-encephalitis of some type other than mumps

Sex	Age (years)	Day of disease	Cells per mm³	Total proteins mg/100 ml	No. of pathologic findings
M	22	2	62	73.5	7
		62	5	35	0
M	49	1	862	54	7
		47	5	34	0
M	53	2	176	44	4
		24	48	42	3
		73	2	37	0
F	56	1	68	65	2
		84	0	40	0
M	54	7	716	123	4
		18	122	47	3
		29	66	49	1
M	19	2	128	60	4
		23	30	49	2
		68	6	34	0
F	7	1	83	27	2
		16	3.8	36	1
		53	0	36	0
F	20	1	443	39	2
		3	56	39	0
		20	6	32	1
		57	0	33	0

Table III, which gives the findings made in eight cases of virus meningo-encephalitis of some origin other than mumps, includes two cases (M 22 and M 49) with unusually many pathologic fractions for virus meningo-encephalitis despite largely normal total protein values and a relatively mild clinical picture. In seven of these eight cases the immuno-electrophoretic pattern of the cerebrospinal fluid became normal within 2—3 months.

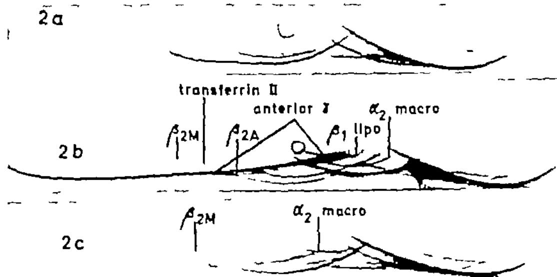


Fig 2 a. Immuno-electrophoretic pattern of a normal cerebrospinal fluid sample. Here and in all following illustrations the preparations were coloured with amido black 10 B.

b. Pattern of cerebrospinal fluid in a patient with bacterial meningitis. The pattern shows e g β_1-lipoprotein, β_{2A} and β_2-macroglobulin α_2-macroglobulin precipitate of serum type, a marked precipitation of the anodic part of the γ-globulin and a poorly developed second transferrin.

c. This preparation from a patient with a Russian spring-summer encephalitis shows e g β_2-macroglobulin and a marked α_2-macroglobulin fraction.

never higher than 19 per cent in the patients with infectious diseases. The paper electrophoretic method thus did not demonstrate any significant differences between the controls and the various clinical groups in this series.

The results from the immuno-electrophoretic study contrast sharply with the meagre paper-electrophoretic findings. Thus "pathologic findings" were demonstrated in all the cerebrospinal fluid samples from the cases of bacterial meningitis, in 86 per cent of the cases of virus meningo-encephalitis and in all the four cases of virus meningo-encephalitis with a normal cell count and normal total proteins.

Of the various pathologic findings in the cerebrospinal fluid the occurrence of β_1 lipoprotein β_{2A} and β macroglobulin fibrinogen α_2-macroglobulin of the type seen in serum, and marked precipitation of the large molecular part of the γ-globulin were common findings in the clinical groups. β_{2B} and β_{2C}-globulin were seen in only a few cases. Transferrin of the type seen in serum, i e without the posterior line or transitional forms of this fraction are rare in virus meningo-encephalitis but are common findings in patients of bacterial meningitis with a high total protein level in the cerebrospinal fluid. Cases with the transitional forms have not been included in table I

This cross-sectional study which was performed in the acute stage of the disease was supplemented by a time-course investigation in several of the patients, most of whom were followed up until the cerebrospinal fluid pattern had become normal. The findings made in

Table II. Number of cells, total proteins, and number of pathologic immuno-electrophoretic findings in cerebrospinal fluid found on repeated examination of 6 patients with mumps meningo-encephalitis

Sex	Age (years)	Day of disease	Cells per mm³	Total proteins mg/100 ml	No. of pathologic findings
M	24	2	400	115	3
		51	2	47	0
F	44	1	98	31	1
		16	22.5	37	0
F	17	1	224	52	2
		63	2.5	29	0
M	7	3	372	35	3
		22	33	33	2
M	6	1	161	42	3
		12	9.4	30	1
F	8	31	171	112	7
		44	134	112	6
		58	24	58	3
		74	5.6	38	5
		130	3	36	2

six cases of mumps meningo-encephalitis followed up in this way are given in table II.

It is clear from the table that the number of pathologic findings tended to decrease with the recovery of the patient. This tendency was, however not without exceptions, pathologic fractions sometimes persisting long after the total protein content and even the number of cells had become normal. Thus, in one case in which the patient (F 8) had recovered and the number of cells and the total protein content had become normal within 10 weeks of onset of the disease, 2 pathologic findings, namely the presence of β_{2A}-globulin and a marked anodic part of the γ-arc, persisted for at least further 2 months.

Table III. Number of cells, total proteins, and number of pathologic immuno-electrophoretic findings in cerebrospinal fluid found on repeated examination of 8 patients with virus meningo-encephalitis of some type other than mumps

Sex	Age (years)	Day of disease	Cells per mm³	Total proteins mg/100 ml	No. of pathologic findings
M	22	2	62	73.5	7
		62	5	33	0
M	49	1	862	54	7
		47	5	34	0
M	33	2	176	44	4
		24	48	42	3
		73	2	37	0
F	36	1	68	65	2
		84	0	40	0
M	54	7	716	123	4
		18	122	47	3
		29	66	49	1
M	19	2	128	60	4
		23	50	49	2
		68	6	34	0
F	7	1	85	27	2
		16	3.8	36	1
		35	0	36	0
F	20	1	445	39	2
		3	56	39	0
		20	6	32	1
		57	0	33	0

Table III which gives the findings made in eight cases of virus meningo-encephalitis of some origin other than mumps, includes two cases (M 22 and M 49) with unusually many pathologic fractions for virus meningo-encephalitis despite largely normal total protein values and a relatively mild clinical picture. In seven of these eight cases the immuno-electrophoretic pattern of the cerebrospinal fluid became normal within 2—3 months.

Table IV. Total proteins and number of pathologic immuno-electrophoretic findings in cerebrospinal fluid found on repeated examination of 3 patients with "encephalitis" without signs of meningitis

Sex	Age (years)	Day of disease	Total proteins mg/100 ml	No. of pathologic findings
M	30	1	30	2
		33	33	0
F	30	6	46	2
		23	25	0
F	46	14	42	2
		30	42	0

Table V. Number of cells, total proteins and number of pathologic immuno-electrophoretic findings in cerebrospinal fluid found on repeated examination of 3 patients with bacterial meningitis

Sex	Age (years)	Day of disease	Cells per mm³	Total proteins mg/100 ml	No of pathologic findings
M	19	1	9,000	179	5
		7	9	44	3
		22	31	47	3
		105	4 3	33	0
M	17	1	18,000	474	6
		5	57	61	4
		19	11	41	1
M	19	1	11 700	390	6
		3	2,500	158	0
		12	15	54	1
		60	35	50	0

The findings made in three cases of meningo-encephalitis without meningeal inflammation are summarized in table IV. As mentioned previously pathologic fractions were found in the immuno-electrophoretic pattern *in all of these* cases. Examination of cerebrospinal fluid obtained one month later showed no such fractions, though none of the patients had then made a complete clinical recovery.

Two of the five cases of bacterial meningitis could be *followed until the immuno*-electrophoretic pattern had become normal (table V). Of the other three patients, two died before normalization of the pattern and one, from whom the cerebrospinal fluid was last obtained 3 weeks after the onset of the disease, refused to co-operate further. The immuno-electrophoretic pattern of the cerebrospinal fluid in that case was not quite normal at this occasion. The most remarkable observation in this small group was the tendency of the number of pathologic fractions to diminish rapidly after institution of adequate therapy.

No correlation was found in the virus cases between the nature of the clinical picture at onset, i. e. elevated body temperature, neck-stiffness, headache etc., and the number of pathologic fractions in the immuno-electrophoretic pattern of the cerebrospinal fluid. In some of the cases with only mild symptoms at onset and only relatively few immuno-electrophoretic abnormalities, the latter nevertheless persisted for a long time. In contrast to what was seen at the onset of the disease, in the further course there was a tendency for the pathologic findings in the cerebrospinal fluid to be correlated with symptoms such as fatigue, headache and reduction of working capacity.

Comments

All of the cerebrospinal fluid proteins discussed above are presumably derived from the blood or are at any rate immunologically identical with blood proteins, since the cerebrospinal fluid proteins

under consideration were precipitated with anti-human and anti-fibrinogen serum and since a given fraction in the cerebrospinal fluid occupied the same position in the immuno-electrophoretic pattern as the corresponding fraction in the blood. It is tempting to assume that the appearance of large-molecular plasma proteins in infectious diseases of the central nervous system is a manifestation of some disorder of the blood/cerebrospinal fluid barrier which normally only permits the passage of small-molecular substances in demonstrable amounts. It has, however also been considered whether the immuno-globulins β_{2A}, β_{2B}, β_{2}-macro β_{2C} and γ-globulin, might be formed locally the cerebrospinal space then being one such site. The appearance of these proteins including marked anterior γ-globulin in the cerebrospinal fluid could then be regarded as a sign of a pathologic condition of the central nervous system, since they did not occur in our controls. Whether these proteins are formed in the central nervous system or whether their presence in the cerebrospinal fluid is a manifestation of a disturbance of the blood/cerebrospinal fluid barrier however remains to be shown. But it seems most likely that these immuno-globulins resemble other pathologic proteins and thus that their appearance in the cerebrospinal fluid in diseases of the central nervous system is a sign of a disorder of the blood cerebrospinal fluid barrier.

In the present investigation no attempt was made to analyse the serum systematically for pathologic protein findings. Such an investigation would, however probably be itself informative, quite apart from the findings made in the cerebrospinal fluid and discussed above. Thus, Clausen, who used agar-gel electrophoresis, in some cases of meningo-encephalitis was able to demonstrate serum fractions not occurring in normals (13).

Summary

In an immuno-electrophoretic investigation of thirty-six cases of virus meningo-encephalitis (including nineteen with mumps meningo-encephalitis) five with bacterial meningitis and four with meningo-encephalitis without signs of meningeal inflammation, and thirty controls consisting of patients without any neurologic evidence of disease and without signs of any serious mental disorder the following observations were made

1 Altogether nine protein findings not demonstrable in the controls were seen in the patients with infectious diseases of the central nervous system. Such findings were regarded as "pathologic."

2 One or more pathologic findings concerning cerebrospinal fluid proteins were seen in 86 per cent of the patients with virus meningo-encephalitis, in spite of the fact that the total proteins and the paper electrophoretic pattern were often normal. Pathologic immuno-electrophoretic findings were demonstrated in all four of the patients with meningo-encephalitis without an increased number of cells or increased total proteins in the cerebrospinal fluid. In the cases of bacterial meningitis the immuno-electrophoretic pattern was usually extremely abnormal

3 The patients with virus meningo-encephalitis showed a wide variety of immuno-electrophoretic abnormalities. In some cases these abnormalities persisted for a long time, even after the cell count and the total proteins had become nor

mal No relationship was found between the severity of the clinical picture at onset of the virus disease and the number of abnormal immuno-electrophoretic findings. During the further course, however some association was discernible between persistent *symptoms* and the presence of pathologic immuno-electrophoretic findings.

Acknowledgements

We wish to thank Mrs. Stina Ohlsson and Miss Margareta Linell for technical assistance.

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'Silent' Mitral Stenosis

By

ELIS MALERS and SVEN-MARTEN SAMUELSON

Mitral stenosis without any clinical signs obviously does not exist. There is always one and there are often several features in the clinical history and the bedside findings in favour of the diagnosis, even if each finding by itself may be non-specific. There may be a positive history such as previous rheumatic fever symptoms from the pulmonary circulation (dyspnoea on effort, cardiac asthma, pulmonary oedema, haemoptysis, recurrent bronchitis) symptoms from the heart itself (tachycardia, atrial fibrillation) systemic embolism and right ventricular failure with peripheral oedema.

Auscultation is usually considered to be the method most used and most reliable in the diagnosis of mitral stenosis. In addition there are often other bedside findings confirming the diagnosis such as palpatory signs of hypertrophy of the right ventricle pulse small in volume, atrial fibrillation, in later stages peripheral cyanosis and the mitral facies. Next in diagnostic value come roentgenological findings, primarily enlargement of the left atrium and calcification of the mitral valve. Electrocardiographic findings are more non-specific but may show signs of hypertrophy of the left atrium and eventually also right ventricular hypertrophy. However even with careful auscultation of the whole precordium at different times, both at rest in the recumbent and left lateral positions and immediately after exercise, there are a few cases where the typical diastolic murmur and the equally typical opening snap of the mitral valve cannot be heard. This seldom occurs in early mitral stenosis but chiefly in later stages with pronounced pulmonary hypertension and an extremely high pulmonary vascular resistance. Such a case without a typical diastolic murmur is usually called a 'silent' mitral stenosis (21, 27).

At the Department of Medicine, University Hospital, Uppsala, we have studied two cases without the typical diastolic murmur both of which have been operated. They both had several features in common, for instance a pronounced, unusual, aneurysmal dilatation of the pulmonary artery.

Case reports

Case 1. A 35-year-old wife with 2 children. No heart disease in the family. No rheumatic fever. Even as a child she became easily tired,

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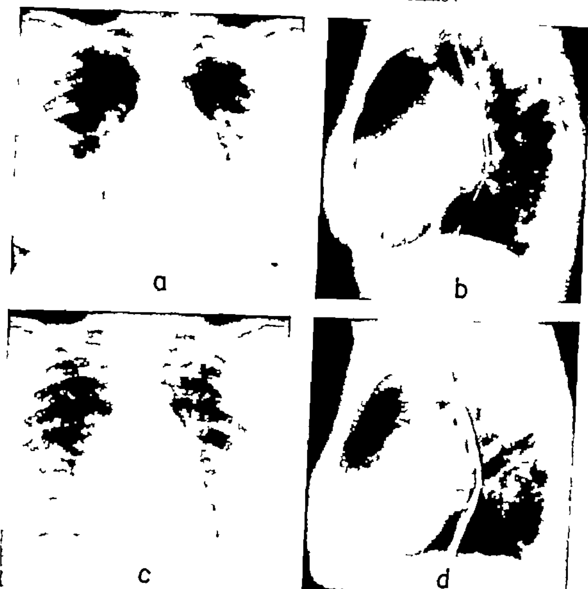


Fig 1 Case No. 1 a) and b) Thoracic roentgenogram in May 1959 before operation. N t the large dilatation of the pulmonary artery the main stem of the pulmonary artery measures a maximum of 70 mm in diameter on the angiocardiographic film) and d) Jan 1961 after operation. The size of the heart is diminished, the pulmonary aneurysm is removed.

did not manage to play games as other children Sometimes cyanotic and acutely dyspnoeic There have also been attacks of syncope. Increasing cardiac symptoms, especially dyspnoea, but also peripheral oedema since the first pregnancy in 1949 Normal delivery however in 1949 and 1952. In November 1958 imminent circulatory collapse in connection with a viral pneumonia.

Physical examination The general condition is good at rest. No cyanosis. No mitral facies. Normal body habitus. No capillary pulsation and no sounds or murmurs over the femoral artery on auscultation. Blood pressure 125/90. Palpation no obviously increased pulsation of th right entricle, apex beat not distinctly palpable Heart sounds (auscultation and phonocardiography) a somewhat accentuated first sound, a faint opening snap over the apex. Over the base of the heart, maximally in the second left ntercostal space at the sternum is heard a systolic murmur of ejection type grad 3 — not conducted to the carotid arteries — and over the same area there is also a *faint*,

high-pitched diastolic murmur of regurgitant type. Over the apex is heard a faint presystolic but no mid-diastolic murmur. Regular rhythm. Time intervals Q-T is 0.08 sec, and T-O S. 0.07 sec.

ECG Sinus rhythm, P mitrale, right axis deviation but otherwise no signs of hypertrophy of the right ventricle. Orthostatic test heart-rate change from 60 to 90/min and depressed ST-segment. Work test does not manage 200 kpm/min in circulatory steady state, final heart rate 145, rate of respiration 78/min. She stops after 4 min because of general tiredness and dyspnoea. ECG marked depression of the ST-segment, mostly over the right ventricle (the patient was under digitalis).

X-ray examination of the heart and the lungs (fig. 1). Total volume 1,070 ml = 670 ml/m body surface, enlargement of right atrium and ventricle, and slightly enlarged left atrium. There is also a large dilatation of the pulmonary artery with increased hilar vascular markings. Tomography: no calcification.

Right-heart catheterization. A moderate pulmonary hypertension at rest, PA 50/27 (mean pressure 35) mm Hg, which during 200 kpm/min after 2 min rises to 108/54 (mean pressure 70) mm Hg. PC mean pressure 17 mm Hg at rest. End-diastolic right ventricular pressure 4 mm Hg. No signs of left-to-right shunt or valvular stenoses of the right heart.

Angiocardiography from the right ventricle. Right ventricle enlarged, pulmonary valves normal. The main stem of the pulmonary artery is considerably dilated and measures maximum of 70 mm in diameter, also the two main branches are obviously widened. The left atrium is slightly enlarged, maximal volume 110 ml (1). The ascending aorta is somewhat dilated.

Left atrial puncture using the method of Björk (4). Pressure 40/23, mean 30 mm. *Angiocardiography.* The mitral cusps are thickened and fused to form a dome, and show diminished mobility. The width of the mitral orifice is 10 mm measured on the film.

Operation May 1959 (V. O. Björk). *Transventricular dilatation of the mitral orifice and excision of the pulmonary aneurysm.* Very tight mitral stenosis, fibrotic thick-walled ring, the orifice measures 6 × 6 mm. With dilatator the ring can be widened to the width of 1 1/2 finger breadths. No insufficiency appears. The aneurysm of the pulmonary artery is also excised. *Histologic examination* of the pulmonary arterial wall: no thrombi. Some small granulomatous collections of lymphohistiocytic elements, localized both in the adventitia and in the media. In the presence of such granulomas the elastic tissue of the media is fragmented. Slight changes of non-specific arteritis.

Postoperative examination Feb. 1961. She feels little improved. She can now manage 200 kpm/min in circulatory steady state with a heart rate of 117 without any symptoms. The auscultatory findings over the pulmonary area are considerably less pronounced, in the apical area as scanty as before. Cardiac catheterization shows lower pressure values in PA, 37/17, mean 26 mm and PC mean 12 mm. During exercise PA rises to 69/33, mean 47 mm and PC to mean 38 mm (uncertain value, however). Cardiac output at rest 3.7 l/min, during work (200 kpm/min) 6.1 l/min. Pulmonary vascular resistance at rest 4 R. Roentgenologically a marked decrease of heart volume, total volume 830 = 510 ml/m² body surface (fig. 1 c—d).

Case 2. A 48-year-old man. No heart disease in the family. No rheumatic fever. As long as he can remember susceptible to tiredness, dyspnoea and palpitations on effort. 'Congenital heart disease' was diagnosed in 1931. Roentgen examination at that time showed a slight enlargement of the heart, a bulging left atrium and pathologically dilated pulmonary artery. Not accepted for military service in 1932; military service for some months in 1941—1942, then exempted. Agricultural work up to 1945, turner up to 1947, since then incapable of work. Pension since 1949. In 1948 acute abdominal pain (mesenteric embolism?), the same year a transitory right-sided hemiplegia (cerebral embolism?). In 1947, 1957 and 1960 bilateral pneumonia. The last 2—3 years more and more marked cardiac symptoms, now and then nocturnal attacks of cardiac asthma. Ankle oedema the last year, needs 3 pillows at night. Continuous digitalis therapy since 1946, diuretic therapy for one year.

Physical examination. The general condition is good at rest. Peripheral cyanosis of lips, tips of the ears and fingers. Faint sounds over

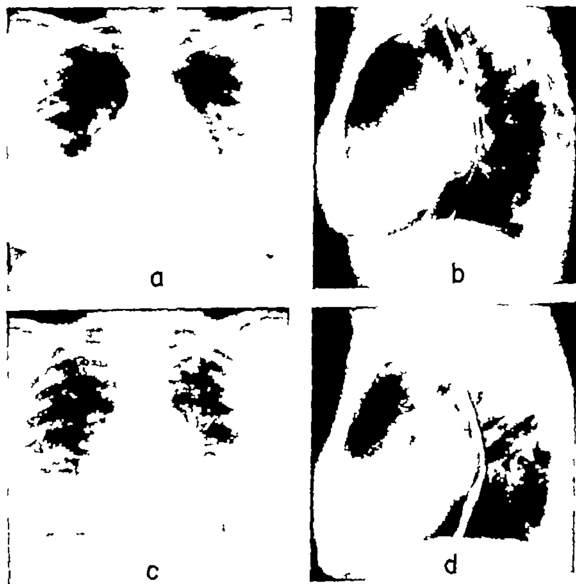


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did not manage to play games as other children. Sometimes cyanotic and acutely dyspnoeic. There have also been attacks of syncope. Increasing cardiac symptoms, especially dyspnoea, but also peripheral oedema since the first pregnancy in 1949 Normal delivery however in 1949 and 1952 In November 1958 imminent circulatory collapse in connection with a viral pneumonia.

Physical examination. The general condition is good at rest. No cyanosis. No mitral facies. Normal body habitus. No capillary pulsation and no sounds or murmurs over the femoral artery on auscultation. Blood pressure 125/90. Palpation no obviously increased pulsation of the right ventricle, apex beat not distinctly palpabl Heart sounds (auscultation and phonocardiography) a somewhat accentuated first sound, a faint opening snap over the apex. Over the base of the heart, maximally in the second left intercostal space at the sternum is heard a systolic murmur of ejection type grade 3 — not conducted to the carotid arteries — and over the same area there is also a faint,



Fig. 3 a) and b). Case No. 2. Thoracic roentgenogram in April 1961 before operation. Note the pronounced dilatation of the pulmonary artery (90 mm in diameter in lateral angiocardiographic projection).

mesenteric embolism, although the patient is adequately treated with dicumarol during the whole stay in hospital.

Operation April 1961 (V. O. Björk). *Transventricular dilatation of the mitral orifice and excision of the pulmonary aneurysm*. The left auricle is filled with thrombotic mass, as large as the distal phalanx of the thumb, on the posterior wall of the left atrium. A tight mitral stenosis with completely calcified orifice is palpable. With dilatator it is possible to widen the ostium to the width of 1 1/2 finger breadths. The whole anterior cusp then consists of a slightly mobile calcified mass, while the posterior cusp is considerably shrivelled. Immediately before the commissurotomy a diastolic pressure gradient of 20 mm Hg is observed; the gradient disappears after the operation. The 'aneurysm' of the pulmonary artery is partly removed.

Histologic examination of the pulmonary arterial wall: thick arterial walls consisting of thick media and abundant elastica of the type seen in the aorta.

Postoperatively the patient gets respirator treatment to reduce his respiratory work; the respirator can be safely removed after one day. A marked improvement is noted after the operation, the patient needs only one pillow at night as opposed to five earlier and experiences less dyspnoea on short walks. However, the patient requires diuretic treatment due to right ventricular failure immediately after the operation.

Discussion

In the cases described the main differential diagnoses were:

1. *Congenital heart disease*. Left-to-right shunt with secondary pulmonary hypertension, for instance an atrial septal defect, could not be excluded. As evidence against an uncomplicated atrial septal defect there was the enlarged left atrium and in case No. 1 also the presence of a faint opening snap. Similarly an atypical patent ductus arteriosus was not excluded, at least in case No. 2 who had a right ventricular hypertrophy. Only the large dilatation of the pulmonary artery was in favour of a pulmonary stenosis or a rare congenital pulmonary aneurysm.

2. *Primary pulmonary hypertension* could possibly explain the clinical picture at least in case No. 2. However, the history was remarkably long and the enlargement of the left atrium was still unexplained.

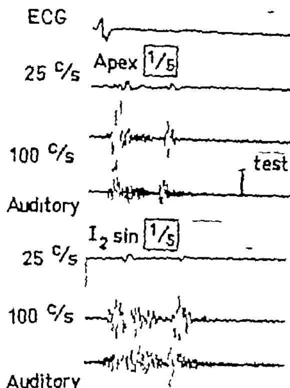


Fig. 2. Case No. 2. ECG lead I and phonocardiogram (nominal frequencies 25 c/s, 100 c/s, auditory). Note the increased Q-I time.

the femoral artery on auscultation and faint capillary pulsation. The liver edge is one finger-breadth below the costal margin. Blood pressure 155/100. Palpation: a slight precordial bulging, somewhat increased pulsation of the right ventricle, apex beat not palpable. Heart sounds (auscultation and phonocardiography): somewhat accentuated first sound over the apex and an accentuated second sound over the pulmonary area. Over the whole heart a systolic murmur of ejection type of maximal intensity (grade 3) in the second and third left intercostal spaces at the sternum; the murmur is faintly heard in the second right intercostal space but not over the carotid artery. Over the base there is also an early-diastolic, high-pitched murmur of regurgitant type, maximally in the third left intercostal space parasternally (fig. 2). No opening snap. Irregular rhythm without pulse deficit. Time interval Q-I is 0.08 sec.

ECG. Atrial fibrillation with a normal ventricular rate, right axis deviation, a high R/S ratio, an increased activation time of the right ventricle and ST-T changes (probably partly dependent on digitalis). A picture of right ventricular hypertrophy.

Work test. Manages 350 kpm/min in circulatory steady state, final heart rate 145, at which the patient gets dyspnoea and ventricular extrasystoles from one focus.

X-ray examination of the heart and lungs. Total volume 2 010 ml = 1 060 ml/m² body surface. Enlargement of the right ventricle and the left atrium. A large dilatation of the pulmonary artery centrally with a rapid decrease in size peripherally (fig. 3 a—b). Tomography: no calcification.

Right-heart catheterization. Pulmonary hypertension at rest, PA 72/37 (mean pressure 51) mm Hg, PC mean pressure 20 mm Hg. End-diastolic right ventricular pressure 0 mm Hg. No signs of left-to-right or right-to-left shunt or valvular stenoses of the right heart.

Angiocardiography from the right ventricle: Right ventricle extremely enlarged. The pulmonary cusps seem to have a diminished mobility. A pronounced dilatation of the main stem of the pulmonary artery (90 mm in lateral projection) and of its two main branches. The left atrium is slightly enlarged.

Retrograde left ventricular catheterization with angiocardiography. Pressure in left ventricle 160/0, in the ascending aorta 160/95. Thus no systolic pressure gradient over the aortic cusps. Angiocardiography: the left ventricle is enlarged, 295 ml (1). The mitral cusps are thickened and form a pronounced dome in both frontal and lateral pictures. The aortic cusps are also thickened and have a diminished mobility; the aortic orifice has the width of about a little finger. A moderate fusiform dilatation of the ascending aorta is also noted.

Thoracic aortography. The whole left ventricle is gradually filled with contrast medium by regurgitation but there is no equality of contrast density between the aorta and the ventricle. About four systoles after the end of the injection the left ventricle is emptied of contrast medium.

Clinical assessment. The patient is accepted, although with hesitation, for operative treatment of the tight mitral stenosis. There is also an aortic stenosis without pressure gradient and a slight to moderate aortic insufficiency. The operation is delayed due to recurrent pulmonary embolism and probably a small



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Histologic examination of the pulmonary arterial wall: thick arterial walls consisting of thick media and abundant elastica of the type seen in the aorta.

Postoperatively the patient gets respirator treatment to reduce his respiratory work; the respirator can be safely removed after nine days. A marked improvement is noted after the operation, the patient needs only one pillow at night as opposed to five earlier and experiences less dyspnoea on short walks. However the patient requires diuretic treatment due to right ventricular failure immediately after the operation.

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In the cases described the main differential diagnoses were:

1. *Congenital heart disease*. Left-to-right shunt with secondary pulmonary hypertension, for instance an atrial septal defect, could not be excluded. As evidence against an uncomplicated atrial septal defect there was the enlarged left atrium and in case No. 1 also the presence of a faint opening snap. Similarly an atypical patent ductus arteriosus was not excluded, at least in case No. 2, who had a right ventricular hypertrophy. Only the large dilatation of the pulmonary artery was in favour of a pulmonary stenosis or a rare congenital pulmonary aneurysm.

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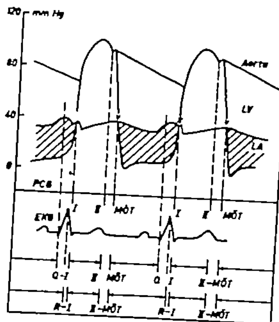


Fig 4 A picture showing the length of Q-1 and 2 O S. (i. e. II MÖT) in relation to the height of the left atrial pressure. An increase of the left atrial pressure causes a longer Q-1 time and a shorter 2-O S time. A decrease of the left atrial pressure causes a shorter Q-1 time and a longer 2-O S. time (7)

3 *Left ventricular failure* after previous myocarditis could not be excluded Ischaemic heart disease was unlikely because of sex (case No 1) and age. In case No 2 there were some clinical signs of aortic insufficiency This disease, however could not explain the whole clinical picture in this case. Similarly rare forms of cardiomyopathy (15) or possibly endocardial fibroelastosis could not be excluded with certainty

4 *Silent mitral stenosis* seemed the most likely diagnosis. The auscultatory findings in the pulmonary area and the pronounced dilatation of the pulmonary artery were not incompatible with this diagnosis. However the presence in case No 1 of a faint atrial-systolic murmur and an opening snap — as a rule not sufficient for the diagnosis of tight mitral stenosis (19 20) — together with a long history could agree with a dominant myocardial factor associated with a slight mitral stenosis (14)

Right heart catheterization, which in the two cases gave elevated pressures in PA and PC as the only finding and angiocardiography from the right ventricle, excluded all other possibilities except for the last mentioned two i. e. silent mitral stenosis or left ventricular failure. Owing to the rather atypical clinical findings and the absence of calcifications of the mitral cusps at tomography a continued examination was indicated However in the two cases there was a delayed first sound which indicated a diastolic pressure gradient over the mitral valve (7 24 30) (fig 4) A choice had to be made between a left atrial puncture and a left ventricular catheterization with pressure measurements and angiocardiography In the first case left atrial puncture with angiocardiography was chosen which verified the diagnosis of tight mitral stenosis In the second case on the suspicion of concomitant aortic insufficiency a retrograde left ventricular catheterization was performed with angiocardiography from the left ventricle and the ascending aorta A normal end-diastolic pressure in the left ventricle and a dome of the mitral cusps (6) verified the diagnosis of tight mitral stenosis. In addition there were also aortic stenosis and insufficiency of slight to moderate degree

Both the cases were operated with successful commissurotomy and excision of their pulmonary aneurysms. The histological picture with clear changes in the vascular walls of the pulmonary artery justified the classification of aneurysm in only one of the cases (8) As expected the operations have given only partial subjective and objective improvement.

In the re-examined case (case No. 1) the pulmonary vascular resistance has probably diminished a little, leading to less strain for the right ventricle. Measurements of flow were not made before operation, so that a more exact comparison has not been possible. In addition the pulmonary aneurysm and the heavily dilated pulmonary artery have become much smaller following operation, leading to diminished risks of some complications.

The occurrence of pulmonary aneurysm in one of the cases, which is very rare (8, 9 11 28) and the large dilatation of the pulmonary artery in the second case is of interest from two points of view. Firstly such a condition may give rise to dyspnoea, haemoptysis and pain through local pressure, secondly it may predispose to thrombus formation leading to secondary pulmonary embolism (3 25). In our cases it has probably also had some haemodynamic significance as a buffer between the right ventricle and the left atrium, leading to only a slight enlargement of the left atrium. The murmurs in the pulmonary area could be explained by these changes of the pulmonary artery (18, 23 31).

The most constant auscultatory finding in mitral stenosis is the diastolic murmur at the apex often preceded by an opening snap. The low-pitched character of this diastolic murmur depends on the comparatively small pressure difference between the left atrium and ventricle in contrast to the murmur and the pressure difference between the artery and the ventricle in aortic or pulmonary insufficiency. The intensity of the murmur depends on the degree of the stenosis as well as the magnitude of the flow. These two factors also decide the duration of the murmur. In general, the rule is that the longer the murmur the tighter the stenosis. But there is a source of error namely tachycardia.

The diastolic murmur may however be of very low intensity even inaudible and of very short duration in a tight mitral stenosis because of other factors. The factors involved may be listed as follows

1) Decreased cardiac output and diastolic atrioventricular flow extremely high pulmonary vascular resistance and right ventricular failure. A high pulmonary resistance (10—30 R) is reported to occur in some 8 per cent in mitral stenosis (32 33). This figure of course, varies in different series depending on the number of advanced cases included. This factor can hardly alone cause the disappearance of the diastolic murmur

2) Change of position of the left ventricle right ventricular hypertrophy thick thoracic wall and emphysema.

3) Alteration of flow through the stenotic valve possibly implying a change of direction of the atrioventricular jet to a more dorsal one (massive thrombi in left atrium (29))

4) Presence of a 'subclinical' aortic valvular disease, of moderate degree especially an aortic insufficiency. This will dilate the left ventricle and may increase the end-diastolic pressure in the left ventricle, and thus cause a change of some of the factors which influence the turbulence of flow i. e. direction of the jet, time course and magnitude of inflow and resonance function of the ventricular wall.

5) Possibly a dilatation of the left ventricle. This has been suggested sometimes to cause the appearance of an apical diastolic murmur even when the valves are normal and may on the other hand cause the attenuation of a stenotic diastolic murmur. A left ventricular dilatation is reported to occur in later stages of isolated

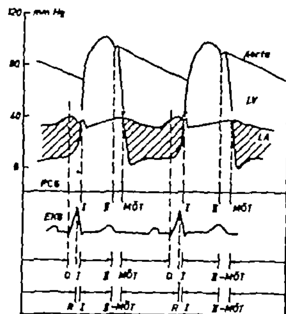


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mitral stenosis (26) A slight aortic valve lesion might contribute to such an effect without *increasing end-diastolic* ventricular pressure.

In 'silent' cases of tight mitral stenosis *some of these factors may be combined* to cause disappearance of the diastolic murmur In our two cases there was still *a flow giving a diastolic pressure gradient* over the mitral valve. In spite of this no apical diastolic murmur could be heard *Contributory factors in our cases could* be right ventricular hypertrophy dilatation of the left ventricle and thrombi in *the left atrium (the last two factors pres*ent in our second really 'silent' case)

The frequency of 'silent' mitral stenosis varies in different series. In *Wood's series* (32, 33) of 300 patients with mitral stenosis there were 2 cases one of which was verified at operation. Among 351 patients Olesen (23) had 6 verified 'silent' cases, thus about 2 per cent but in addition 21 probable cases who had died but not been subjected to autopsy In another *Danish material* of surgical origin described by Baden (2) there were 2 such cases out of 167 patients. In a French series *(10) 'silent' mitral stenosis occurred* in about 6 per cent *(12 cases* out of 206 7 of which were verified at operation and 5 at *section)* *In our last five year series* of cases examined cardiologically there were 184 cases of mitral stenosis. Of these, 54 were *combined* with mitral insufficiency and 26 with aortic stenosis of varying degree. Thus, out of 104 cases of isolated mitral stenosis we encountered the present 2 'silent' cases of these only the second case was really 'silent' *However this was* not a real case of isolated mitral stenosis as a slight to moderate aortic insufficiency with slight ventricular dilatation was present, too. The diastolic pressure gradient over the mitral valve was significant, though, according to our pressure measurements preoperatively and during operation. In a few cases of the mixed group (combined with dominant aortic or mitral insufficiency and/or aortic stenosis) we were also able to diagnose a slight mitral stenosis due to left heart catheterization and puncture. In these cases the mitral stenosis was of *minor* degree and of little clinical importance.

Of some interest in this connection is a *third case, where the auscultatory find*ings at rest were at variance with the other clinical findings (the result of the *right heart catheterization included)* the latter agreeing rather well with the diagnosis of mitral stenosis. Left heart catheterization *confirmed a diastolic pressure* gradient of about 20 mm Hg over the mitral valve. A faint diastolic murmur of *filling or stenotic type* over the apex was only present immediately after a comparatively heavy work load (200 kpm/min) and with the patient in a left lateral position. The difficulty of hearing the diastolic murmur typical of mitral stenosis was possibly explained by the pronounced obesity of this patient.

*Nowadays it is possible to get an ac*curate preoperative diagnosis by means of left heart catheterization even in 'silent' cases of mitral stenosis (5 16 17) Another method which ought to be of diagnostic value in some cases of 'silent' mitral stenosis may be the so-called ultrasonic cardiogram (UCG) (12 13) The difficulty of avoiding a false positive diagnosis without a complete investigation can be illustrated by mentioning a case from our clinic, wrongly diagnosed as mitral stenosis, which went to commissurotomy operation without a previous left heart catheterization. Many clinical findings, including a long history with for instance *pulmonary oedema* and

cerebral embolism, agreed with the diagnosis of mitral stenosis but a diastolic murmur a delayed and accentuated first heart sound, an opening snap and calcification of the cusps were lacking. At operation the patient had completely normal mitral cusps but a pronounced fibrosis of the myocardium. A Beck operation was made assuming the diagnosis to be ischaemic heart disease.

In conclusion, a tight isolated mitral stenosis is rarely present if the typical diastolic murmur is lacking. Probably more than one factor has to be present at the same time with diminishing effect on the intensity and duration of the murmur to cause absence of this murmur. An aortic insufficiency of slight to moderate degree may sometimes be involved without causing disappearance of the diastolic pressure gradient over the mitral valve by increasing the end-diastolic pressure in the left ventricle. An aortic insufficiency of such a degree may be difficult to diagnose at the bedside.

The diagnosis of tight 'silent' mitral stenosis (absence of stenotic diastolic murmur and of opening snap) should be suspected in the presence of

1) *Clinical history and findings* Previous rheumatic fever cardiac symptoms, especially in a typical chronological order pulmonary oedema, haemoptysis atrial fibrillation, systemic embolism, right ventricular failure and typical bedside findings. Signs of another dominating valvular or myocardial disease should not be present.

2) *Roentgen findings* Enlarged left atrium, signs of pulmonary hypertension, but especially calcifications corresponding to the mitral level. These should be pronounced, should be localized to the cusps, not merely scanty calcifications of the annulus.

3) *ECG-findings* P mitrale or atrial fibrillation right axis deviation right ventricular hypertrophy

4) *Phonocardiographic findings* Delayed first heart sound usually recognized as accentuated on auscultation.

Right-heart catheterization is of value in all cases fulfilling these criteria even if the diagnosis already seems to be evident. The degree of pulmonary hypertension and pulmonary vascular resistance can then be confirmed, and postoperative treatment with respirator can be planned in advance. In some other cases, especially those lacking calcification of the cusps and a delayed first heart sound, the examination must be supplemented with left heart catheterization to exclude a left ventricular failure on the basis of a myocarditis or ischaemic heart disease.

Summary

In a series of 184 patients with mitral stenosis, out of which 104 were 'isolated mitral stenoses of significance' there were 2 cases without an apical mid-diastolic murmur. However one of these cases also had a slight to moderate aortic insufficiency with a normal end-diastolic left ventricular pressure but an enlarged left ventricle. This condition may possibly be a contributory factor underlying the rare absence of the diastolic murmur in such cases. Both cases had a pronounced dilatation of the pulmonary artery one being a true aneurysm, a very rare phenomenon.

If the diagnosis of tight mitral stenosis is suspected from the clinical findings in the absence of the typical diastolic murmur it can be confirmed by catheterization of the left side of the heart. This investigation is necessary if pronounced calcification, an opening snap and a delayed first heart sound are lacking.

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Xanthurenic Acid Excretion Studies in Anemic and Non-anemic Carriers of the Fish Tapeworm

By

MARTTI OKA and V V E. LEPPÄNEN

The fish tapeworm (diphyllobotrium latum) competes with the host for vitamin B_{12} causing a deficiency of this vitamin and, in some cases, pernicious anemia (Bonsdorff 1947 1956 1959) Carriers of fish tapeworm have also been found to excrete lowered quantities of thiamine, pantothenic acid (Markkanen, Kalliomäki, Oka, Mustakallio and Brummer 1960) and folic acid (Markkanen, Brummer and Savola 1961) suggesting a deficiency of these vitamins.

There are no previous reports on the nutritional status of vitamin B_6 in the carriers of fish tapeworm. We have therefore studied this subject by measuring the urinary excretion of xanthurenic acid in fish tapeworm carriers before and after a loading dose of L-tryptophan

Vitamin B_6 is required for the metabolic breakdown of tryptophan. It acts, in the form of pyridoxal phosphate as a coenzyme of kynureninase (Braunshtein, Goryachenkova and Pashkina 1949 Mason and Berg 1952, Wiss 1952) If this degradation of the kynurenine compounds is inhibited by the administration of the vitamin B_6 antagonists, excess quinoline compounds — kynurenic and xanthurenic acid — are formed from them (Price Brown and Larsson 1957) The metabolic derangement can be corrected by the administration of vitamin B (Wachstein and Lobel 1954 Price et al.) Lepkowsky Roboz and Haagen-Smit (1943) were the first to show increased xanthurenic acid excretion after a tryptophan load in urine of vitamin B_6 deficient rats. The same observation was made later in man (Greenberg, Bohr McGrath and Rinehart 1949 Glazer Mueller Thompson, Hawkins and Vilter 1951) and the measurement of xanthurenic acid in urine after a loading dose of tryptophan was proposed as a sensitive indicator of vitamin B_6 nutrition (Wachstein and Gudaitis 1952)

Material and methods

The material comprised 15 cases infected by fish tapeworm. Six of these had megaloblastic anemia, which was confirmed by bone marrow examination Two had iron deficiency anemia without megaloblastic features

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Table I Xanthurenic acid excretion in anemic and non-anemic carriers of the fish tapeworm

Case no.	Age	Sex	Hemoglobin g/100 ml	Red cells mill./mm	Bone marrow	Xanthurenic acid excretion µmoles/24 hours		
						Before tryptophan	After tryptophan	After tryptophan + B_6
1	55	F	7 1	1 73	Megaloblastic	11	47	15
2	65	M	5.4	1.50	Megaloblastic	23	22	21
3	51	M	9.6	2.06	Megaloblastic	8	19	14
4	67	F	10 1	3.10	Megaloblastic	12	21	15
	60	F	7 6	1.87	Megaloblastic	9	12	7
6	46	M	12.3	3.5	Megaloblastic	19	25	12
7	20	F	9.4	4 31	Iron deficient	23	51	26
8	50	M	10.8	3.51	Not examined	19	35	5
9	54	M	14 4	5 09	Not examined	29	47	27
10	58	M	15.5	5.28	Not examined	29	39	32
11	36	M	15.3	4 96	Not examined	14	23	15
12	37	M	15.8	5 02	Not examined	12	17	11
13	53	F	13.7	4 63	Not examined	13	27	Not tested
14	35	F	11 6	4 12	Not examined	19	17	Not tested
15	43	M	14.5	5.15	Not examined	9	14	Not tested
					Mean	16.6	27 7	16.2
					Range	8–29	12–39	3–32

and the rest had no anemia. Twelve hospitalized cases in which no organic diseases were found served as controls.

Xanthurenic acid was estimated from 24 hour urine specimen by the method of Rosen Lowy and Sprince (1951) Toluene was used as a preservative. The estimations were performed on three consecutive days. The first day was a control day On the morning of the second day a dose of 2 g of L-tryptophan (9 8 m moles) was administered. On the morning of the third day 100 mg of pyridoxine (Benadon "Roche") was given intramuscularly 30 minutes before the administration of a second dose of 2 g of L-tryptophan.

All medication was discontinued one day before the experiment. The natural L-isomer of tryptophan and the dose used were selected on the basis of Price's studies (1958)

Results and discussion

The results are presented in tables I and II There was no significant difference in the xanthurenic acid excretion between anemic or non-anemic carriers of fish tapeworm and the healthy controls either before or after the loading dose of L-tryptophan. Administration of B_6 vitamin before the second loading dose of L tryptophan brought the xanthurenic acid excretion near the basal level in those cases in which the effect of B_6 was tested

Our results indicate that fish tapeworm carriers do not suffer from vitamin B_6 deficiency neither does it have any role in the development of their pernicious anemia. We know that pyridoxine is necessary for hemsynthesis (Schulman and Rinehart 1957) and that pyridoxine responsive anemias occur in man (Harris, Whittington Weisman and Horrigan 1956, Jones and Hutt 1961 Dawson Leeming Oelbaum, Pengelly and Wilkinson 1961) As a rule, pyridoxine-

responsive anemia is hypochromic and microcytic but one exceptional case has been reported (Maier 1957). Here the bone marrow was megaloblastic but there was no response to liver extract, folic acid or vitamin B_{12}. The xanthurenic acid excretion after tryptophan load has been abnormal only in some of the cases of pyridoxine-responsive anemia tested (Dawson et al.)

These data on pyridoxine-responsive anemia also support the view that vitamin B_6 deficiency is not a contributory factor in the development of megaloblastic anemia in fish tapeworm carriers. However the results of this study do not preclude the possibility that the fish tapeworm could use this vitamin in its own nutrition without disturbing the vitamin B_6 nutritional status of the host.

Summary

The urinary excretion of xanthurenic acid was studied in 15 carriers of fish tapeworm before and after a loading dose of 2 g of L-tryptophan. Six of the cases had megaloblastic anemia. The control material comprised 12 healthy subjects.

The basal xanthurenic acid excretion and the rise in xanthurenic acid excretion after tryptophan load did not differ significantly between anemic and non-anemic carriers of fish tapeworm or between the fish tapeworm carriers and the healthy controls. Intramuscular injection of 100 mg of vitamin B_6 before the administration of a second dose of L-tryptophan lowered the xanthurenic acid excretion to basal level in both the fish tapeworm carriers and the controls.

The conclusion is that fish tapeworm carriers do not suffer from vitamin B_6 deficiency

Table II Xanthurenic acid excretion in healthy controls

Case no.	Age	Sex	Xanthurenic acid excretion µmoles 24 hours		
			Before tryptophan	After tryptophan	After tryptophan + B_6
1	22	M	18	36	Not tested
2	47	M	23	26	Not tested
3	32	F	7	19	Not tested
4	28	F	4	11	Not tested
5	42	M	17	37	Not tested
6	31	M	6	13	Not tested
7	19	M	13	36	Not tested
8	30	M	26	65	Not tested
9	22	F	9	0	Not tested
10	27	F	18	28	Not tested
11	39	M	19	25	15
12	62	M	16	43	20
		Mean	14.7	29.1	17.5
		Range	4–26	10 65	15 20

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From the Neurological Department N (Head: Hans Haugsted) Odense County and City Hospital, Odense Denmark

Familial Episodic Adynamia

By

TH. O. IVERSEN

Familial episodic adynamia was first described by Sagild in 1954. So far it has been found only in one family in Denmark which had originally immigrated from Sweden. From Sweden three families have been reported and Gamstorp (1956) found that one of the Swedish families was related to the one living in Denmark. Since no case of familial episodic adynamia has been reported previously in a purely Danish family it seems justified to report the following case, recapitulating first the most important characteristics of the disease.

Familial episodic adynamia has many features in common with familial periodic paralysis. In both, the clinical picture is characterized by attacks of pareses in striated muscles. These attacks may occur at any time of the day apparently with a preference for the morning, but never during the night. The duration of each episode is generally short, from a few minutes up to about one hour. They vary in intensity and extent from mild paresis in one limb to severe paralysis of all limbs. The muscles of the trunk, neck, and especially the face may be involved as well. Tendon reflexes in the affected muscle groups are usually weakened or absent. In each patient, the severity of the attacks may vary but even during severe attacks there is – unlike periodic paralysis — as a rule some reserve strength in the affected muscle groups. During the attack the surface sensibility is normal.

The frequency of these episodes varies within wide limits from one case to the next. Some patients have daily attacks, while others have only a few in a year. The attacks are more common in winter than in summer.

The most important provoking factors are cold and rest, especially when the latter has been preceded by physical exertion. As stated above, the attacks appear to have a tendency to occur late in the morning, often combined with a feeling of hunger but the blood sugar level during the attacks is normal. An attack may be induced experimentally by the administration of potassium, and

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in such experiments the similarities to and differences from periodic paralysis are most evident. In periodic paralysis the attacks are associated with hypopotassaemia which may be induced experimentally by administration of glucose while potassium can arrest an incipient attack. In familial episodic adynamia the serum potassium level is normal or slightly elevated during the attacks which may be induced by administration of potassium, while glucose prevents the provoking effect of potassium. In his provocation experiments Sagild (1959) used potassium in doses ranging from 0.25 to 1.50 mEq/kg. He found patients with familial episodic adynamia to give abnormal responses to these quantities. In part, they developed paretic attacks whose intensity was to some extent proportional to the dosage of potassium, and in part they showed marked elevations of the serum potassium level (to a maximum of approx. 3 mEq/l) during the provoked attacks. In his control series corresponding provocation experiments gave no or only a slight increase in the serum potassium level, and there were no signs of pareses.

The protective effect of glucose appears to be reliable with 1.5—2 g/kg.

Electrocardiograms during the attacks show elevated T waves in all leads. Electromyography shows signs of a reduced number of active muscle fibres and increased muscular irritability.

The pathogenesis of the disease — i.e. of the attacks — is unknown. Gamstorp assumed that they were due to loss of potassium from the cells to the extracellular space, but this explanation can hardly be sufficient. Experimental results indicate that accumulation of potassium at the motor end plate takes place in connection with the attacks.

The prognosis is favourable. Unlike periodic paralysis, the individual attacks are generally mild and short lasting and no fatal cases have been observed. There is no specific therapy. A diet low in potassium and high in carbohydrate has been given through several months, but without any influence upon the frequency of the attacks. Moreover calcium, which appears to shorten the potassium-induced attacks, has been tried, but also unsuccessfully. On the other hand, the attacks decrease in frequency if the patients avoid the known provoking factors, viz. physical exertion, cold, and hunger.

According to Gamstorp the inheritance is dominant with complete penetrance but there does not seem to be any indication for special eugenic measures.

Case history

Our patient was a 57-year-old man, a former carpenter now innkeeper. In 1913 he had been admitted to the Odense County and City Hospital with injury to the right kidney. In 1921 rib fracture. About the age of 20 the patient was an amateur boxer and a few days after a knock-out he developed difficulty of controlling his legs and repeated generalized convulsions within 24 hours. He was in hospital for 6 weeks and was discharged completely symptom-free.

In 1927 he had been admitted with melaena, and the next year he had a gastric resection.

His present trouble has existed for 35—40 years. For many years it was in the form of paroxysmal severe fatigue, increasing to generalized weakness. The paroxysms varied in duration from 5 to 30 minutes. During mild attacks the patient noted only weakness of both legs, during more severe attacks also in the arms. The intensity increased rapidly within the first few minutes, often so that the patient had to lie down. However, he had never fallen, never had convulsions, headache, dizziness, or impaired consciousness.

When he had rested for 10—20 minutes, the attack subsided in most cases gradually and in between the attacks he had no complaints at all. None of the attacks had been in the form of paralysis, only intensive fatigue and feeling of great weakness. Even during the severe attacks the patient had noticed that some reserve strength remained in the muscles. This may be exemplified by an attack which came on when he was crossing crowded street. It was so severe that he had to lie down in the middle of the street, but his fear of being run over mobilized so much reserve strength that he was able to drag himself to the pavement.

In the course of time, the disease has altered somewhat. The individual attacks are no longer so characteristic. In particular he no longer has completely symptom-free intervals. Now one attack has a tendency to overlap with the next, the patient feeling tired all the time, having frequent exacerbations relieved only by moderate improvement without complete remissions.

He has noticed a number of provoking factors, primarily work, especially in stooping position. Rest is helpful, but if it lasts too long it is difficult for the patient to get going again. In cold weather the attacks are particularly severe, and the patient has also noted an increased frequency of attacks in connection with hunger. He rarely has attacks immediately after meals.

In 1954 he was admitted to the neurological department of the Odense County and City Hospital. Physical examination showed no abnormality. Laboratory findings: Serum potassium before an attack 4.5 mEq/l and after an attack 4.1 mEq/l. A potassium analysis was carried out in connection with an exercise experiment. The value increased from 3.5 mEq/l to 3.8 mEq/l. A glucose tolerance test showed an increase from 115 mg/100 ml to 290 mg/100 ml within half an hour but decrease to the initial value within an hour. Continued determinations during the subsequent three hours revealed unchanged values between 95 and 140 mg/100 ml.

Biopsy specimen of muscle was normal.

Urine: No albumin or sugar.

From March 3rd—26th, 1960, he was again in hospital. Physical examination showed normal conditions, also neurologically. However his gait was striking, with a slight suggestion of paresis and tendency for giving way in the knees.

Laboratory findings: E. S. R. 7 mm/hour. Hb. 96 %, R. B. C. 4.42 mill., W. B. C. 5,500, differential count showing a normal distribution. Urine without albumin or sugar. Blood pressure 170/100. E. C. G. normal.

Serum chlorid 105 mEq/l, serum sodium 136 mEq/l, serum potassium 4.6 mEq/l. Fasting blood sugar 82—95 mg/100 ml. A glucose tolerance test showed an increase from 100 mg/100 ml to 250 mg/100 ml within half an hour and fall to the initial value within 1 1/2 hour. Determinations of the blood sugar during the subsequent two hours showed values around 65—70 mg/100 ml. The fasting test for 24 hours showed fluctuations in blood sugar from 70 to 100 mg/100 ml.

Radiography of the skull, chest and urinary tract showed no abnormality. Radiography of the stomach also showed no abnormalities apart from the sequelae to gastric resection with gastro-entero-anastomosis by the Billroth I method. Passage through the intestinal tract normal.

During the first days after admission the patient had few minor incipient attacks during which the serum potassium, E. C. G. B. P. and blood sugar remained normal.

On March 17 a potassium tolerance test was carried out with a dosage of 1 mEq potassium per kg body weight. The patient was fasting, and the result was as follows:

	Potassium mEq/l	Dynamometer	Pareses
Before the test at			
8.30 a. m.	4.6	130	0
8.45 a. m.	5.8	110	0
9.00 a. m.	6.4	130	0
9.15 a. m.	6.5	100	+
9.45 a. m.	6.9	80	+
10.15 a. m.	6.3	140	0

+ in the pareses column indicates that the affected muscle groups showed moderate, but unmistakable paresis.

The patient felt tired and weak as during spontaneous attack, and at 10 clock he was unable to raise himself to sitting position.

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fatigue and giving way in the knees when getting up after sitting for a while. He takes good care to have his meals regularly since otherwise there is tendency to minor attacks.

Discussion

The diagnosis of episodic adynamia was based on the description of the disease and the serum potassium, electromyographic, and electrocardiographic changes as well as mild pareses during provocation experiments with potassium. As already mentioned, administration of glucose prior to a potassium tolerance test prevents the provoking effect of potassium. The latter test failed, presumably because the patient had a moderate dumping syndrome.

Several members of the patient's family have symptoms of episodic adynamia. We were particularly keen to perform tolerance tests on III 4 who had a history of rather severe attacks, but he refused in no uncertain terms. However the symptoms show such uniformity and similarity to episodic adynamia that it is justified to assume that the disease is familial.

The University Institute of Human Genetics, Copenhagen, has traced the family back to 1820 without finding any connection with Sweden. All the members of the family within the past 150 years had been born on the isle of Funen so this appears to be the first case in a purely Danish family.

Summary

The first case of episodic adynamia in a purely Danish family is described. Several members of the patient's family have symptoms so uniform and similar to episodic adynamia that the condition must be assumed to be familial.

An account of the most important characteristics is given. In many respects, the clinical picture has features in common with periodic paralysis, and like the latter the disease is characterized by attacks of pareses. Experimentally the attacks may be induced by administration of potassium. During the provoked attacks investigations showed marked increases in the serum potassium level, EMG signs of a reduced number of active muscle fibres and increased muscular irritation. Preceding administration of glucose prevents the provocative action of potassium.

It is stated that the prognosis is favourable. No specific therapy is known but the frequency of the attacks may be reduced by avoiding the provoking factors viz. physical exertion, hunger and cold.

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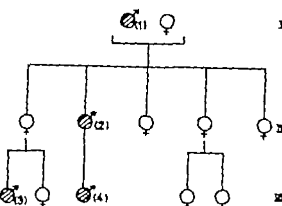


Fig 1 Pedigree of the family

On March 19th another potassium toler ance test was performed this time using 1.5 mEq potassium per kg body weight. This test too was carried out on an empty stomach, and the result was as follows

	Blood sugar	Potassium mEq/l	Dynamometer	Pareses
Before the test at		5 1	120	0
8 45 a m	76	5 8	130	+
9 00 a. m.	72	6.8	130	+
9 15 a m.	95	6 5	120	0
9 30 a. m	76	7 0	130	0
10 00 a. m	84	6.8	120	+
10 30 a. m.	82	7 0	130	0

During this latter test electromyography was performed at the hours stated Before the test it showed no abnormality at the end of 15 minutes a marked loss of potentials, at the end of 30 minutes a moderate loss, and thereafter only a slight loss until the conclusion of the test

Throughout the test the patient reported that he was feeling very tired and weak as in a spontaneous attack, and it was only with the utmost difficulty that he could raise himself to a sitting position.

A provocation test with potassium after administration of glucose (2 g per kg) proved a failure, as the intake of glucose was followed by nausea, sweating, and repeated copious watery vomiting The serum potassium level remained normal, 4 4—4.5 mEq/L

Family history (cf. fig 1)

I 1 P H born in 1869 died in 1948 gardener The data were given by II 2. Said to have come home from work several times after having collapsed The symptoms occurred in attacks during which he had allegedly been unable to stand or walk. Never actual paralyses. Tired more easily than normal, and attacks were said to have occurred especially after sitting still for some time. Had always been considered weak, unfit for hard physical work. Never admitted to hospital.

II 2 The present case.

III 3. J L., born in 1937 a student of economics. Since the age of 15 a few attacks of marked paroxysmal fatigue and a tendency for the knees to give way The attacks have always been short, a maximum of a few minutes, not definitely related to meals, and with no particular accumulation in winter time. They occur particularly during walking but subside when the patient goes on walking He avoids major physical strain. Is left handed. Since the age of 7 a tendency to stuttering said to have been provoked during the war by bombing of a house where the family were living

III 4 P H born in 1927 book-keeper From the age of 10—15 frequent attacks of left-sided headaches accompanied by nausea and vomiting sometimes several times a week. Since the age of 15—17 a tendency to paroxysmal severe fatigue in the arms and particularly the legs. These attacks were described as massive fatigue in the limbs which he could lift only with the utmost difficulty They would occur particularly late in the morning or in the afternoon, never immediately after meals. Resting accentuated the attacks. "When I noticed that I was getting tired while working and therefore stopped, it would be really bad" Some tendency to improvement in recent years, but the patient deliberately avoids any physical exertion. Still a tendency to

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Subvalvular Aortic Stenosis of Muscular Type

By

P. Fritz Hansen, H. Gotta Davidsen and J. Fabricius

Distinction is at present made between the following types of stenoses of the outflow tract of the left ventricle: 1) valvular stenosis, 2) subvalvular fibrous, discrete aortic stenosis, and 3) subvalvular or infundibular stenosis of muscular type. To these types should be added the supravalvular stenosis, which is a proximally situated type of coarctation. The existence of the two first-mentioned types has been recognized for several years, while the muscular type was closely studied only in recent years. As early as 1907 Schmincke (13) described two autopsy cases, in which the outflow tract of the left ventricle was markedly narrowed because of hypertrophy of the myocardium. Apart from this, no attention was paid to this disorder until 1957 when Brock (5) described the finding of a left ventricular outflow tract obstruction depending on muscular conditions. Since then, the anatomical and haemodynamic conditions in this disease have been described several times (1, 3, 4, 6, 8, 11, 12, 15, 16).

A precise preoperative diagnosis of the type of the aortic stenosis has become necessary for the selection of cases suitable for surgery and for the planning of the surgical procedure.

During the last two years, we have diagnosed subaortic muscular stenosis in four patients, three males and one female. The case histories of these four patients are presented below. A brother of one of the patients suffered probably from the same disorder and consequently his case history is presented too.

All the patients were examined by means of combined suprasternal puncture and direct percutaneous puncture of the left ventricle (10). By this technique simultaneous pressure recordings are obtained from the left ventricle and the aorta, as well as from the left atrium and the left ventricle. The puncture needles employed for the direct cardiac puncture do not allow the introduction of a catheter. Consequently this method furnishes no information as to the site of a stenosis. Retrograde catheterization of

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Fig I Case I Pressure tracings from left ventricle subaortic chamber and aorta showing a pressure gradient in the left ventricle. No gradient across the aortic valve.

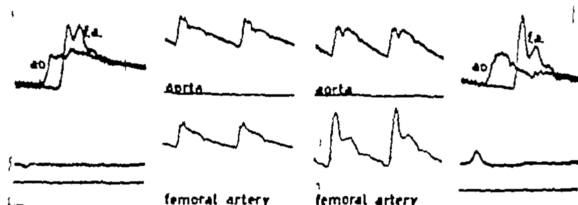


Fig. 2. Pressure tracings from aorta and femoral artery

the left ventricle was therefore performed by means of a 100 cm long polyethylene catheter inserted via the brachial or the femoral artery by Seldinger's percutaneous technique (14) With the catheter tip placed in the left ventricle, Urografin® 76 % was injected for contrast examination of the left ventricle. Following the ventriculography pressure recordings were performed during withdrawal of the catheter from the ventricle. The cardiac output was determined by the dilution technique, using I^{131} in iodinated human serum albumin or Cardiogreen as indicators.

Case reports

Case I E. D A 30-year-old male with no family history of heart disease. In a routine examination at the age of 26, a heart murmur was detected. During the three years prior to admission, the patient had developed increasing dyspnoea on exertion and slight precordial pain. He was of a healthy appearance without cyanosis or dyspnoea at rest. The apex beat was felt in the 2nd intercostal space to the left of the midclavicular line. At the apex and along the left sternal border a systolic thrill was palpable A grade 4 systolic murmur and a faint proto-diastolic murmur were heard at the apex The intensity of the murmurs diminished towards the left sternal border and was of grade 2 in the 2nd right intercostal space they were poorly heard over the neck vessels. The second aortic sound was inaudible and the peripheral arterial pulse was normal. The arterial blood pressure was 115/70 mm Hg Electrocardiogram showed sinus-rhythm, left axis deviation, left ventricular hypertrophy and strain. On X ray examination of the chest the transverse cardio-thoracic ratio was 0.48, but there was an isolated prominence of the left ventricle Tomography revealed no calcification of the aortic valve.



Fig. 3. Case 1. Selective left ventriculography Above Left lateral projection. Below Antero-posterior projection. In systole (right) the ventriculogram shows pronounced constriction in two places. The arrows →← point to narrow passage between the aortic leaflet of the mitral valve and the ventricular wall. The arrow ⟼ points to constriction formed by the greatly hypertrophied septum which bulging into the ventricular lumen. In diastole (left) there is no visible constriction.

Retrograde catheterization of the left ventricle revealed systolic pressure gradient of 157 mm Hg between the left ventricle and a subvalvular chamber while there was no pressure gradient across the aortic valve. (Left ventricle 260/17 mm Hg chamber 103/17 mm Hg aorta 103/69 mm Hg.) A characteristic pressure curve from the aorta was obtained, showing rapid upstroke and an early maximum followed by gradual fall in pressure (cf. figs. 1 and 2) Cardiac index was 2.4 l/min/m.

Selective left ventriculography showed the lumen of the left ventricle to be hourglass-shaped, due to pronounced bulging of the septum into the outflow tract of the ventricle. The obstruction varied with the contraction phase of the heart, being most pronounced during systole, while there seemed to be broad passage during diastole. The thickness of the left ventricular wall was 25 mm. There was slight regurgitation of the contrast medium into the left atrium. The width of the aorta was normal, and also the function of the aortic valve appeared normal (fig. 3)

Case 2, E. V. A 20-year-old male. A brother of this patient had suffered from congenital heart disease and died suddenly at the age of 20 (cf. case 2). There was no history of rheumatic fever or tonsillitis. At a routine examination at the age of 13 his heart was found to be enlarged. At his first admission to this hospital at the age of 14 the patient was asymptomatic. He had a heaving apex beat in the 5th intercostal space in the mid clavicular line. A grade 2 systolic murmur and proto-diastolic blowing murmur were heard at the apex The intensity of both murmurs diminished towards the left sternal border and they were poorly audible over the aorta. The systolic murmur reappeared over the neck vessels, and the aortic and pulmonary second sounds were equal. The peripheral pulse was of the water-hammer type. Electrocardiogram showed sinus-rhythm and left ventricular hypertrophy and strain. Right heart catheterization revealed pulmonary-artery wedge pressure of 13 mm Hg. The pressures in the pulmonary artery and the right ventricle were 25/12 mm Hg and 25/0 mm Hg, respectively Angiocardiography with injection of contrast medium into the right atrium showed normal conditions. The conclusion was drawn that there was a left-sided heart disease of unknown

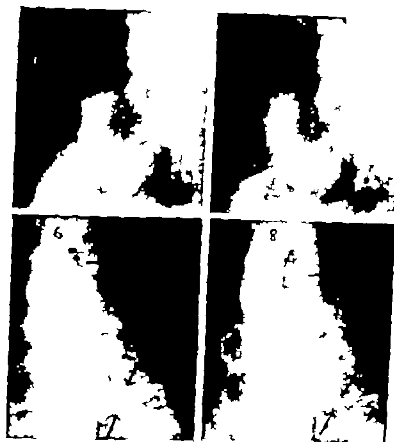


Fig 4 Case 2. Selecti e left ventriculography Systole (right) hows a pronounced narrowing of the ventricular lumen caused by the hypertrophied septum I diastol (left) there is a wide ventricular lumen.

type. The second admission took place when the patient was 17 years old. His subjective condition was unchanged and good but now the electrocardiogram showed increased S—T deviations. A thoracic aortography was performed, which revealed a normal aortic valve and normal width of aorta.

In 1960, the third admission took place, the patient being 20 years of age. Now he suffered from exertional dyspnoea and precordial pain on effort. Examination of the heart revealed the apex beat in the 5th intercostal space 3 cm to the left of the mid clavicular line the impulse being diffuse and heaving There was a marked pulsation in the neck vessels. A grade 4 harsh systolic murmur and a diastolic gallop rhythm were heard at the apex The intensity of the murmurs diminished towards the base of the heart. Over the aorta the systolic murmur was of grade 2 and there was also a blowing diastolic murmur The aortic sound was louder than the pulmonary second sound. The peripheral arterial pulse was collapsing Electrocardiogram showed greater S—T deviations than previously X-ray examination of the chest showed a slightly enlarged transverse diameter of the heart and isolated prominence of the left ventricle. The transverse cardio-thoracic ratio was 0.54 By combined suprasternal and left ventricular punctures a systolic pressure gradient of 80 mm Hg was measured between the left ventricle and the aorta. (Left ventricle 180/8 mm Hg aorta 100/70 mm Hg) A gradient of 14 mm Hg was found between a subvalvular chamber and the aorta. Cardiac index was 3.5 l/min/m The pressure curve from the aorta showed a similar configuration as in case 1 (fig 2) Selective left ventriculography showed pronounced hypertrophy of the left ventricular wall. Four cm below the aortic valve the lumen was considerably narrowed during the systole on account of bulging of the septum, while a broad lumen was present during diastole. The contrast medium did not enter the left atrium, and the aorta was of normal width (fig 4) On right heart catheterization in 1961 the following pressures were recorded Pulmonary artery wedge pressure 16 mm Hg



Fig. 5. Case 3. Simultaneous pressure recordings from left ventricle and aorta. Description: See text.

pulmonary artery 39/14 mm Hg, conus of the right ventricle 40/2 mm Hg, right ventricle 46/2 mm Hg, right atrium 3 mm Hg. Presumably the slight fall in pressure in the right ventricle indicates the presence of an infundibular pulmonary stenosis produced by the hypertrophied ventricular septum.

Case 2 a, *M. F.* Case 2 a is brother of case 2. He was first admitted to this hospital in 1951 at the age of 19 years. His symptoms had made their first appearance one year previously with precordial pain and syncope on running. Physical examination revealed diffuse heaving and systolic thrill over the precordium. The apex beat was palpable in the 5th intercostal space in the anterior axillary line. A harsh systolic murmur and a diastolic gallop rhythm were heard at the apex. The intensity of the murmurs diminished towards the base, but they were again heard over the neck vessels, where there was vigorous pulsation. The aortic and pulmonary second sounds were equal. Over the aorta a faint diastolic murmur was heard. The capillary pulse was visible on the forehead and under the nails, and a pistol-shot sound was heard over the peripheral arteries. Musset's sign was present. The peripheral arterial pulse was collapsing. Arterial blood pressure was 110/60 mm Hg. Electrocardiogram was normal with QRS-axis of + 30 degrees. Exercise with a load of 600 kg/min for 7 minutes was accompanied by precordial pain and followed by pathological deviation of the S—T segment and negative T waves. X-ray examination of the chest showed normal size and form of the heart shadow. Right-heart catheterization showed normal conditions. The clinical diagnosis was aortic stenosis and incompetence. One year after his discharge from the hospital, the patient suddenly dropped dead while ploughing. No autopsy was performed. The clinical findings in this case were similar to the findings in the brother S. N. and it is considered probable that they suffered from the same type of heart disease.

Case 3. *E. B.* A 43-year-old Icelandic woman without any family history of heart disease. There was no past history of rheumatic fever, diphtheria or tonsillitis. She had previously been in good health apart from a goitre. During the last four years prior to the admission to this hospital in 1960, the patient had suffered from exertional dyspnoea, precordial pain and oedema of the ankles, which had disabled the patient during the last six months. There had never been syncopes. On physical examination the apex beat was found in the 5th intercostal space in the midclavicular line. There was a grade 4 systolic murmur in the 4th left intercostal space, but no diastolic murmur. The intensity of the murmur diminished to grade 1 towards the base. The aortic and pulmonary second sounds were equal. The peripheral arterial pulse was normal. Electrocardiogram showed sinus-rhythm, QRS-axis of 0 degrees, and left strain. X-ray examination of the chest showed isolated left ventricular prominence and a cardiothoracic ratio of 0.55. Tomography of the heart revealed no calcification of the aortic valve. By suprasternal and left ventricular punctures a systolic pressure gradient of 148 mm Hg was measured between the left ventricle and the aorta. (Left ventricle 252/6 mm Hg, aorta 104/67 mm Hg.) Retrograde arterial catheterization, during which it was

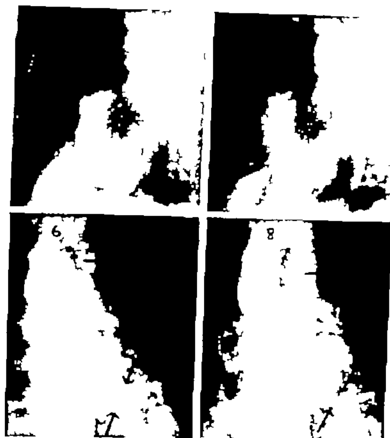


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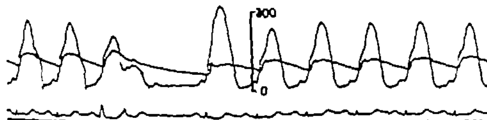


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impossible to pass the subvalvular stenosis, revealed a *pressure gradient* of 35 mm Hg across the aortic valve. Cardiac index 4 0 l/min/m²

Selective left ventriculography showed *the lumen of the left ventricl* to be hourglass *shaped with a long muscular* prominence situated 4 cm below the aortic valve. The stenosis was most pronounced during the ventricular systole. There was marked hypertrophy of the ventricular wall, particularly of the septum which bulged *into the lumen*. Slight regurgitation of contrast medium to the left atrium was observed The ascending aorta was of normal width and the function of the aortic valve appeared normal

Case 4 J L. A 47 year-old male without any family history of heart disease. At the age of 10 he had diphtheria, and during adolescence he suffered frequent attacks of tonsillitis. No history of rheumatic fever Since childhood he had suffered from exertional dyspnoea, but he had been able to perform hard physical work with no essential complaints until the age of 37 In 1950 he was admitted to another hospital, where cardiac auscultation showed normal conditions. *The arterial blood pressure* was 125/70 mm Hg and the electrocardiogram revealed a QRS-axis of 0 to + 30 degrees and negative T waves in lead I During the preceding 10 years, the patient had experienced exertional dyspnoea and precordial pain, and during the last two years these symptoms had been *incapacitating* The patient was first admitted to this hospital in 1955 Now there was a systolic murmur of *grade 1 to 2 along* the left sternal border and of lower intensity over the aorta. At this admission and at a *subsequent* admission in 1958 the diagnosis was left ventricular failure of unknown cause At the third admission in 1960 a systolic murmur was present at the apex. No murmur was audible in the 2nd right intercostal space or over the neck vessels. The second aortic sound was normal. The peripheral arterial *pulse normal*. Electrocardiogram revealed atrial fibrillation and left ventricular hypertrophy and strain. X ray examination of the chest revealed a transverse cardiothoracic ratio of 0.60 and prominence *of the* left ventricle. Calcifications of the mitral valve were found at tomography Right-heart catheterization revealed a slightly increased pressure in the left atrium. The pressure tracings from the right ventricle showed dip-and plateau, the configuration described in *constrictive pericarditis* and various myocardial diseases (9) Pulmonary artery wedge pressure 15 mm Hg By suprasternal and left ventricular punctures a systolic pressure gradient of 102 mm Hg was measured between the left *ventricle and aorta. The pressure tracing from the left atrium* did not indicate mitral disorder As regards the configuration of the aortic pressure curve see *fig 2* Cardiac index *1.9* l/min/m²

At retrograde catheterization of the left ventricle the catheter failed *to* enter the apex *of th* ventricle, but was passed into the left atrium. During withdrawal the catheter passed directly through the subvalvular chamber and the high pressure obtained by the ventricular puncture was not recorded. No *pressure gradient* was found across the aortic valve Contrast examination of the left ventricle revealed pronounced diffuse hypertrophy The apex region of the ventricle was poorly opacified. In the middle of the left ventricle an obstruction appeared during systole, while during diastole there was a good width in this region. There was some regurgitation to the left atrium. Aorta was of normal width, and the aortic valve showed normal function.

Comment

In our four cases, the diagnosis of subvalvular aortic stenosis of mainly muscular *type* was made by intracardiac pressure tracings and by selective left ventriculography In *all the patients* a considerable pressure gradient was found across a stenosis situated below the aortic valve, while gradients across the aortic *valve were absent* or only slight In all cases selective left ventriculography revealed a marked obstruction *of the infundibulum of the* left ventricle during systole. During diastole, however no obstruction was present (figs. 3 and 4) In *all the patients the* aortic pressure curves

showed a characteristic configuration with a rapid upstroke and an almost rectangular shape (fig. 2). This configuration has been described previously in several cases where a diagnosis of subaortic muscular stenosis was verified at autopsy or surgery (5, 8, 11, 15, 16).

Discussion

The aetiology of subaortic muscular stenosis remains obscure. Brock (5) assumes that systemic hypertension may be the causative factor of the development of a subaortic muscular stenosis, but this course has been demonstrated only in one case, and none of our patients have, to the best of our knowledge, previously suffered from hypertension. Subaortic muscular stenosis may develop in cases with valvular aortic stenosis (12) just as the secondary infundibular stenosis develops in valvular pulmonary stenosis. Familial occurrence of subaortic muscular stenosis has been described previously (1, 4) and in our series are included two brothers with heart diseases, probably of identical nature. Presumably some developmental defect is responsible in such cases. According to observations in the literature, it seems reasonable to describe two types of subaortic muscular stenosis, one explained by diffuse myocardial hypertrophy (1), the other being produced by an asymmetric hypertrophy of the interventricular septum (8, 15, 17). On the basis of the selective ventriculographies, our case 4 seems to be of the first group, while the remaining cases are of the type with asymmetric septal hypertrophy. A combined form of fibrous and muscular subvalvular aortic stenosis with a small fibrous ridge attached to heavily hypertrophied myocardium has been described by Brachfeld and Gorlin (3).

Obstruction of the right ventricular outflow tract on account of septal hypertrophy in subaortic muscular stenosis has been described by Goodwin et al. (8) and was demonstrated in one of our patients (case 2).

Regurgitation of the contrast medium to the left atrium as seen in three of our patients might be ascribed to a mitral incompetence, but may also occur in cases with competent valves if extra-systoles appear during the examination.

Diagnosis

Our first three cases presented a uniform clinical picture, all of them having a systolic murmur with its highest intensity along the left sternal border, with lower intensity in the second right intercostal space, and also audible over the neck vessels. A proto-diastolic murmur was heard at the apex in two of the patients. The electrocardiogram showed signs of left hypertrophy and strain and X-ray examination revealed enlargement of the left ventricle, normal ascending aorta and no signs of calcification of the aortic valve. These signs, which correspond to those previously described in the literature (1, 3, 4, 8, 12, 15, 16) are of some diagnostic value. In the fourth of our cases there was only a very faint systolic murmur along the left sternal border, in spite of the fact that there was a systolic pressure gradient of 102 mm Hg in the left ventricle. The electrocardiographic and radiologic findings, however, were similar to those in the other cases. The presence of a loud murmur should, consequently, not be considered a constantly occurring sign in subaortic muscular stenosis. The aortic pressure curve is in our opinion of the greatest diagnostic value. The charac-

teristic pressure curve with a rapid upstroke, an early maximum and an almost rectangular shape was found in all our four patients and has been described in several cases diagnosed during surgery or at autopsy (3 8, 11 15 16) This configuration of the pressure curve is not similar to the anacrotism found in valvular or discrete subvalvular aortic stenosis. Furthermore, we have not previously observed this type of pressure curve among 700 tracings obtained from direct puncture of the ascending aorta The haemodynamical basis of the characteristic pressure curve can probably be explained as follows At the commencement of the isotonic contraction of the ventricle there is unimpeded passage to the aorta and consequently the pressure in this area increases rapidly i e normally During the continued ejection phase, the hypertrophied myocardium causes an obstruction of the outflow tract so that the aortic pressure will not increase in spite of a further rise in ventricular pressure. Hence the degree of the outflow tract obstruction depends on the force of the ventricular contraction This is illustrated by fig 5 which presents a nearly normal configuration of the aortic pressure curve during a ventricular extrasystole, i e with low ventricular pressure there is no sign of a pronounced stenosis in the outflow tract. In contrast to this finding the first contraction following the compensatory pause is characterized by a high ventricular pressure, a lower pulse pressure and a more rapid upstroke in the aorta. Brockenbrough and Braunwald (7) emphasized this phenomenon as a reliable sign in diagnosing the muscular type of subvalvular stenosis as in both the valvular and the discrete type of subvalvular stenosis the aortic pulse pressure varies directly with the left ventricular systolic pressure. Pressure curves from the peripheral arteries (fig 2) in 3 of our cases had two peaks, namely a tall first peak, a systolic dip and a lower second peak. Similar pressure tracings are found in patients suffering from combined aortic stenosis and incompetence.

The following case history serves to illustrate the diagnostic problems

The patient was a 30-year-old male H C., who was admitted to this hospital for complaints of syncopes and dizziness. His sister died suddenly at the age of 20 from congenital heart disease of unknown type On physical examination a grade 2 systolic murmur was heard along the left sternal border but there was no diastolic murmur The peripheral arterial pulse was normal. The electrocardiogram showed signs of left hypertrophy and strain. X-ray examination of the chest demonstrated slight generalized enlargement of the heart and a cardio-thoracic index of 0 52 Right heart catheterization showed normal conditions. Selective left ventriculography showed an obstruction of the outflow tract of the left ventricle during systole resembling that found in the other four patients. However heart punctures revealed no gradient between the left ventricle and the aorta (left ventricle 120/17 mm Hg aorta 120/80 mm Hg) This patient thus had a marked hypertrophy of the left ventricle but no obstruction was caused by the hypertrophy

This case history together with the history of case 4 where only a faint systolic murmur was heard shows that a precise diagnosis cannot be made clinically and that the evidence of an obstruction of the outflow tract at the ventriculography does not in itself substantiate the diagnosis of subaortic muscular stenosis. The presence of a stenosis should be determined by means of intracardiac pressure recordings performed both distally and proximally in the left ventricle

and in the aorta. By means of ventriculography it is possible to get an idea of the length and site of the obstruction and its relationship to the ventricular contraction phase.

Björk et al. (2) demonstrated the presence of an organic subvalvular aortic stenosis by ventriculography. Morrow and Braunwald (12) performed selective left ventriculography in a case of subaortic muscular stenosis and found conditions which agree with our findings. Soulié et al. (16) performed ventriculography in 4 patients, but they found the picture described above only in one of the cases.

Most workers (3, 4, 6, 8, 12) recommend that attempts at surgical correction should not be made for subvalvular muscular obstruction. Goodwin et al. (8) however have successfully resected the hypertrophied myocardium in a case of septal hypertrophy, but this technique can hardly be applied in all cases. None of our patients have been subjected to surgical treatment.

Summary

Four cases of subaortic muscular stenosis are reported. The patients, 3 males and 1 female, were examined by means of a combined heart puncture technique, retrograde catheterization of the left ventricle and selective left ventriculography. The same condition was probably the cause of death in a brother of one of the patients examined. The clinical, electrocardiographic and haemodynamical findings were fairly uniform in three of the patients, while the fourth patient differed from the others in having only a faint systolic murmur. A characteristic configuration of the aortic pressure curve, together with contrast examination showing signs of systolic obstruction in the outflow tract of the left ventricle, is considered to be extremely important for establishment of the diagnosis. It is concluded that the diagnosis cannot be made clinically and that the ventriculography is of diagnostic value only in connection with pressure recordings.

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The Effect of Triparanol on the Serum Lipid Pattern in Hypercholesteremic Patients

By

Aarne Konttinen

Triparanol has been shown by a number of investigators to depress the serum cholesterol level (e. g. Blohm et al. 1959, Oaks et al. 1959, Gould et al. 1959, Mackenzie & Blohm 1959) and this property has led to its use in attempts to counteract atherosclerosis. The effect of this compound is to inhibit the synthesis of cholesterol at the level of desmosterol (24-dehydrocholesterol). However, this latter sterol seems to accumulate in the serum during treatment with triparanol (Steinberg et al. 1961). The effect of the accumulation of desmosterol in the organism in respect to atherosclerosis still requires clarification. Without drawing conclusions at this stage of the investigation as to whether triparanol counteracts atherosclerosis, it may be assumed that this compound is of practical aid in depressing an elevated serum cholesterol level.

The published data support the view that triparanol has a greater depressing effect on serum cholesterol than on serum phospholipids (Blohm et al. 1959, Hollander et al. 1960). In studies in which cholesterol fractions have been included in the program, interest has been focused on the amounts of free and esterified cholesterol in the serum. In these studies the decrease in total cholesterol has been shown to occur at the expense of the esterified fraction (Waddell 1960, Ruskin & Ruskin 1960). The present study will show the effect of triparanol (Mer 29) on serum total cholesterol and phospholipids, and on the cholesterol content of the α- and β-lipoprotein fractions separated by paper electrophoresis.

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Material and methods

The present study was carried out on a series of 37 hypercholesteremic patients. These patients were known to have high serum cholesterol levels (over 350 mg %). Of the patients, who were followed during a period of twelve weeks, 26 were females and 11 males. Their ages ranged from 31 to 72

Mer 29 and placebo tablets were kindly supplied by Merrell-National, London.

Of the lipoprotein fractions β-cholesterol decreased highly significantly ($P < 0.001$) with 250 mg/day of triparanol, but the increase in dosage to 750 mg/day caused no further clear decrease in serum β-cholesterol ($P < 0.05$). In the α-cholesterol fraction the decrease was highly significant with 250 mg of triparanol daily and there was a further and highly significant decrease ($P < 0.001$) when the dosage was increased to 750 mg/day.

Discussion

Serum total cholesterol was found in the present study to decrease by over 20 per cent on a dosage of 250 mg triparanol per day. As there was no simultaneous fall in serum phospholipids the cholesterol/phospholipid ratio decreased considerably during the treatment. This can be held to be an effect relevant to atherosclerosis, if it is conceded that a rise in this ratio contributes to atherosclerosis, a view held by many investigators (e.g. Gertler et al. 1950, Jackson & Wilkinson 1952, Oliver & Boyd 1953). On the other hand, it should be noted in this connection that reduction of the serum cholesterol/phospholipid ratio has been reported to be without effect on the prognosis in coronary heart patients (Oliver & Boyd 1961).

A conspicuous feature in changes of the serum lipids studied is the decrease in α-cholesterol. Although the absolute reduction is greater for β-cholesterol, the relative decrease is even clearer in α-cholesterol, as is seen on a semilogarithmic scale in the figure. During the treatment with the larger dose, 750 mg triparanol per day, the decrease is greater in α-cholesterol than in other lipids. In previous studies α-cholesterol has been demonstrated to be very stable. In dietary studies, for instance, where a lowering of serum cholesterol has occurred, it has been achieved at the expense of β-cholesterol, α-cholesterol remaining unchanged (Bronte-Stewart et al. 1956, Anderson et al. 1957, Keys et al. 1957, Farquhar & Sokolow 1958). In population studies α-cholesterol has likewise been noted to be very similar in population groups which have been shown to differ from each other in total cholesterol and β-cholesterol (Bronte-Stewart et al. 1955, Keys et al. 1958, Brunner & Lobl 1958). In connection with triparanol medication, information concerning the behavior of α-cholesterol is lacking. The only relevant report concerns the changes observed in three monkeys, in which serum α-cholesterol was found to decrease during triparanol administration (Blohm et al. 1959).

It is difficult to interpret the significance of the decrease in α-cholesterol. In relation to atherosclerosis it is interesting to note that some investigators have found a higher β/α ratio in atherosclerosis than in controls (Nikkilä 1953, Oliver & Boyd 1955). The decrease in α-cholesterol observed in the present study is conceivably a reflection of impairment of liver function, as there is evidence of a decrease in α-cholesterol in liver diseases (Klein & Franken 1955). Although triparanol seems to be very free of side effects (Goyette & Elder 1960, Lisan et al. 1960, Ruskin 1960, Achor et al. 1961), there have been several observations suggestive of disturbances of liver function during triparanol medication (Hollander et al. 1960). From the results obtained in the present study, however, it is not possible to draw conclusions as to the significance of the decrease in the α-cholesterol fraction.

Table I Mean values and standard errors of the mean of serum lipids in 37 hypercholesteremic patients for different determinations during the study Between A and B the patients received 250 mg triparanol per day Between B and C the dose was 750 mg per day and between C and D placebo tablets were given. Between D and E 250 mg triparanol per day was again given

Lipid	A	B	C	D	E
Total cholesterol	377.7 ± 10.94	303.5 ± 8.35	286.0 ± 9.44	362.1 ± 9.40	325.7 ± 9.52
α-cholesterol	63.0 ± 3.31	52.9 ± 3.04	44.4 ± 2.57	55.8 ± 2.24	54.0 ± ..71
β-cholesterol	314.8 ± 11.98	250.5 ± 8.85	242.3 ± 9.63	306.3 ± 10.07	72.0 ± 9.33
Phospholipids	338.5 ± 10.44	337.6 ± 8.53	307.9 ± 8.43	329.2 ± 6.64	303.5 ± 3.31

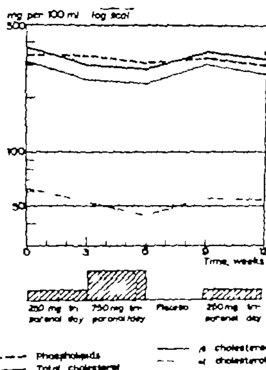


Fig 1 Changes in serum lipids during triparanol medication.

years. Of these patients 21 had coronary heart disease, 4 diabetes mellitus and th rest essential hypercholesteremia. Some of the patients were taking some drug such as digitalis, nitroglycerin or meprobamat. These drugs were not changed during the study The patients were also requested not to change their diet during the investigation period.

Serum samples were collected five times, at intervals of three weeks, as is seen from the figure. The total cholesterol (Anderson and Keys 1956) and the total phospholipids (Fiske and Subbarow 1925) were determined and paper electrophoresis was done in a barbital buffer as described earlier (Konttinen 1959) On the α and β-lipoprotein fractions so separated, the cholesterol content was determined The drug and placebo periods are seen in the figure, as well as the dosage of the compound during the different three-week periods. During the first three weeks the dose was 250 mg daily and during the next three weeks 750 mg daily After that period the patients received placebo tablets for three weeks, and in the last three-week period 250 mg triparanol was given as at the beginning of the study

Results

As seen from table I the serum total cholesterol decreased from 377 7 mg per cent to 303 5 mg per cent during the first three weeks, when the patients were receiving 250 mg triparanol daily This decrease is highly significant ($P < 0.001$) On the other hand, during the same period there was no change in phospholipids. When the dosage was increased to 750 mg/day there was a further fall in total cholesterol ($P < 0.02$) With this dosage the decrease in phospholipids was also significant ($P < 0.02$) When placebo tablets were given, there was a return of serum lipids towards the initial values. A new decrease took place when triparanol therapy was resumed.

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Summary

A decrease in serum cholesterol has been demonstrated in patients with hypercholesteremia during treatment with triparanol (Mer 29). In the 37 patients studied the mean drop was from 377.7 mg% to 303.5 mg% in three weeks with a dose of 250 mg triparanol per day. During the same time there was no decrease in serum phospholipids. When the dose was increased to 750 mg/day there was a further decrease in total cholesterol (from 303.5 to 286.0 mg%) and at the same time in phospholipids (from 337.6 to 307.9 mg%).

The decrease in serum cholesterol is shared by the α- and β-cholesterol fractions, separated with the aid of paper electrophoresis. The decrease in the lipid fractions studied seems to be clearest in α-cholesterol, usually a very stable lipid fraction.

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Haemophilia in Sweden

IV Hereditary Investigations

By

Olof Ramgren, Inga Marie Nilsson and Margareta Blombäck

Haemophilia in Scandinavia was first reported by Thal (35) in Denmark in 1829. During the late 19th century and the first three decades of the 20th, numerous accounts were given of cases of this disease in the Scandinavian countries (cf. 2, 32). In 1943 Andreassen (2) published an extensive report of 63 families with haemophilia in Denmark, and 1944 Sköld (32) published a similar review of 60 haemophilic families in Sweden.

New concepts on the pathogenesis of haemophilia were introduced by the finding of Pavlovsky (23) in 1947 that plasma from one haemophiliac could correct the clotting defect in the blood from another. This aroused fresh interest in the study of haemophilia, and investigations on the subject were started in all Scandinavian countries. In 1960, reviews on haemophilia in Denmark and in Finland were published by Sjolin (31) and by Ikkala (15) respectively.

During the period 1955—1960, a continuous investigation has been made of the haemophiliacs in Sweden. The results of coagulation studies on 176 Swedish haemophiliacs (19), of investigations of the carrier state (20) and of the symptomatology of haemophilia A and B (27) have been published earlier. The present paper deals with hereditary investigations of haemophilia in Sweden.

Case material and methods

The investigations were started as a follow up of Sköld's aforementioned study (32) and his 60 haemophilic families were reinvestigated. In order to trace new haemophiliacs,

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Table I. Classification of Swedish haemophili families by level of AHF or B factor

Haemophilia type	No. of families	No of families with 2 or more members tested
A severe	63	15
A moderate	24	4
A mild	28	8
A total	115	27
B severe	13	5
B moderate	10	3
B mild	9	4
B total	32	12
Total	147	39

Table II. Relationship between haemophiliacs in whom coagulation studies were made

Relationship	Generations	No. of relationships tested
Brothers	I	26
Uncle — nephew	II	8
Cousins		3
Grandfather — daughter son	III	3
Second cousins		2
Cousin — cousin son		6
Second cousin — second cousin son	IV	1
Cousin — cousin grandson		1

agulation defect the relationship between the tested members is listed in table II. The haemophilic defect was tested in 26 sibships (brothers) in which the haemophilic gene was the same, and had been transmitted in only one generation. In 13 relationships, the haemophilic gene had been transmitted independently in two generations, and in 11 relationships in 3 generations. In 2 relationships the gene had been handed down in 4 generations. The AHF or B factor values were similar within each of the relationships with heredity in one, two and three generations, i. the disease was of the same degree of severity in all the affected members tested. Thus, in families 13 18 27 31 32 and 54 (heredity in 3 generations) the AHF or B factor was at the same level in all cases in each family In the two families with heredity in 4 generations (nos. 22 and 57) the AHF values did not vary by more than 1 per cent.

It is also evident from our earlier papers (19 20) that all the tested members of the same family — haemophiliacs, as well as definite, probable and potential carriers — had the same type of coagulation defect.

In family 150 with haemophilia B of moderate degree, we found a low AHF level (between 39 and 37 per cent of normal) in all five members tested, i e two haemophiliacs and three definite carriers. In family 107 (haemophilia A of mild degree) we found a low factor V level in the only haemophiliac tested. No combined coagulation defects were found in any of the remaining 145 families, in which tests were made in 187 haemophiliacs and 117 definite, probable or potential carriers.

Positive heredity in Swedish haemophilic families

As shown by the pedigrees (28) and table III, we tried to follow the families for at least four generations. In families 97 and 119 the parents were unknown, so that the family history could not be

the hospital reports to the Royal Swedish Medical Board for the years 1942—1957 were surveyed, and the available case records examined. The haemophiliacs or their relatives received a questionnaire about their family relations, and further information about the families was obtained from the parish registers.

In the previous study of the coagulation defects in Swedish haemophiliacs (19) they were classified into three groups, according to the plasma level of AHF (factor VIII) or B factor (factor IX) *i. e.*,

Severe haemophilia A and B AHF or B factor < 1 per cent of normal

Moderate haemophilia A and B AHF or B factor 1—4 per cent of normal

Mild haemophilia A and B AHF or B factor 5—25 per cent of normal

The clinical picture was compared with the results of the classification in 176 haemophiliacs. The three groups of haemophilia A and B, classified according to the degree of severity were found to be characterized by the following clinical features. (As pointed out in the previous paper (27) moderate haemophilia cannot be distinguished from the severe or mild form by the clinical features alone.)

Severe haemophilia. The symptoms generally appear during the first two years of life, and the diagnosis is usually obvious at the first visit to hospital. Repeated haemarthroses occur in ankle, knee, elbow and wrist joints at an early age, and result in impaired joint function after the age of 10 Moreover severe changes in the joints often give rise to moderate or severe disablement. Large subcutaneous, intramuscular and retroperitoneal haematomas appear spontaneously or after slight trauma. Episodes of haemorrhage from the renal and/or gastrointestinal tract are common. They require frequent hospitalization and often regular transfusions of blood or plasma. The coagulation time is appreciably prolonged *i. e.*, to more than 30 minutes, whereas the bleeding time is normal.

Moderate haemophilia. The first symptoms generally appear before 8 years of age. Haemarthroses are less frequent than in severe haemophilia and, as a rule, are restricted to a few joints, usually ankle, knee and elbow They do not lead to impaired function of the joints before middle age. Spontaneous haematomas in the subcutis, muscles and retroperitoneum are rare. Sporadic episodes of renal or gastrointestinal haemorrhage occur but require hospitalization only when the bleeding is profuse. Blood or plasma transfusions are usually required in the presence of haemarthroses and renal or gastrointestinal haemorrhage. The coagulation time is prolonged, ranging from 10 to 45 minutes.

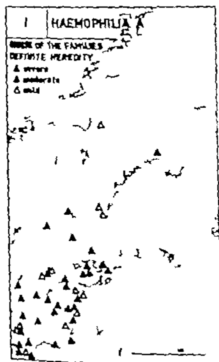
Mild haemophilia. In half of the cases the initial symptoms appear before 8 years of age, and in one fourth of them in adolescence. Haemarthrosis occurs in not more than half of the cases, and only after moderate or severe trauma. It is restricted to one or two joints, and does not lead to impaired joint function. The bleeding episodes take place after tooth extraction or in connexion with minor or major surgery or only as repeated renal or gastrointestinal haemorrhages. Hospitalization is rare, and blood transfusions are needed only when bleeding is profuse. The coagulation time may be prolonged, but is within the normal range in half of the cases.

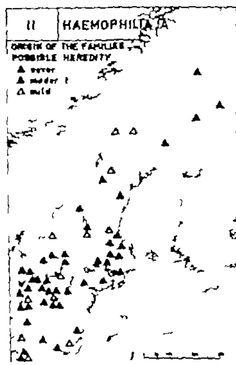
The 33 families not classified by coagulation studies were divided into clinically severe or mild forms of haemophilia, as judged by the clinical features and coagulation time. This was the only possible way of classifying the 23 families studied by Sköld in which there was no living haemophiliac. In two families (nos. 9 and 147) the type of haemophilia could be established by coagulation studies on definite or potential carriers. The potential carrier in family 147 has now given birth to a son with severe haemophilia A.

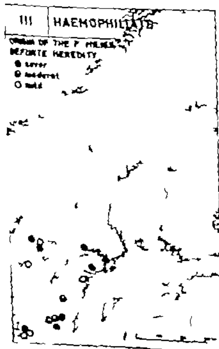
In all, 180 families were studied. The pedigrees with short case histories of established haemophiliacs are given in a supplement (28) As already stated, coagulation studies were performed in 147 of these families.

Coagulation defects in Swedish haemophilic families

The coagulation status of Swedish haemophilic families has been presented in an earlier paper (19) A summary of the results is given in table I together with some supplementary values Two or more members of 39 families were tested. All the affected members of the individual family showed the same co-


I
HAEMOPHILIA A


II
HAEMOPHILIA A
POSSIBLE HEREDITY


III
HAEMOPHILIA B

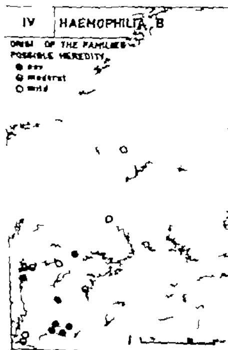

IV
HAEMOPHILIA B
POSSIBLE HEREDITY

Table III Heredity number of generations

Haemophilia type	Total no. of families	No. of generations followed								No. of generations with positive heredity					
		1	2	3	4	5	6	7	8	1	2	3	4	5	6
		No. of families								No of families					
Classified by coagulation test															
A severe	63	1	—	4	30	17	9	2	—	17	17	14	13	1	1
A moderate	24	3	—	1	10	7	3	—	—	10	6	4	3	1	—
A mild	28	2	—	3	10	12	1	—	—	7	4	11	5	1	—
A total	115	6	—	8	50	36	13	2	—	34	27	29	21	3	1
B severe	13	—	—	1	5	5	1	—	1	1	6	1	3	1	1
B moderate	10	—	1	1	6	2	—	—	—	4	3	1	2	—	—
B mild	9	—	—	—	4	4	1	—	—	1	1	4	3	—	—
B total	32	—	1	2	15	11	2	—	1	6	10	6	8	1	1
Total A + B	147	6	1	10	65	47	15	2	1	40	37	35	29	4	2
Clinical form															
Severe	104	1	—	6	51	27	15	3	1	28	27	22	23	2	2
Moderat	34	3	1	2	16	9	3	—	—	14	8	6	5	1	—
Mild	42	2	—	3	16	18	3	—	—	10	3	15	11	1	—
Total	180	6	1	11	83	54	21	3	1	52	40	43	39	4	2

traced. In family 132 the family history was also unknown since the proband's parents came from Esthonia. Family 98 could not be followed as both parents were oligophrenic. Families 130 134 and 136 were unwilling or unable to cooperate in the hereditary investigations. Totally 162 of the remaining 173 families could be followed for at least four generations (table III)

We found a positive heredity in 127 of the 173 families, i e in 73 per cent By positive heredity we mean that the haemophilic gene can be traced for at least two generations we have also regarded a family as having a positive heredity when the mother of the haemophiliac was a probable carrier in whom we found a low level of AHF or B factor

It was shown in an earlier investigation (20) that a low level of AHF or B factor in a probable carrier affords strong evidence that the woman in question is, in fact, a carrier

Origin of the haemophilic families

By following the haemophilic families for four or more generations, we were able to trace their geographical origin back to about 1850 The population of Sweden was then situated mainly in rural districts, and before 1850 was stationary in relatively limited regions. As seen from maps I—IV both haemophilia A and B with definite or possible heredity are evenly distributed over the country in relation to the population density One

Table V. Total number of living and dead haemophiliacs in Swedish haemophilic families

Haemophilia type	No. of families	No. of living and dead haemophiliacs in each family													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
A severe	63	26	16	9	1	4	2	3	—	2	—	—	—	—	—
A moderate	24	13	2	7	1	—	—	—	—	—	—	—	—	1	—
A mild	28	8	6	6	3	2	—	1	1	—	—	1	—	—	—
A total	115	47	24	22	5	6	2	4	1	2	—	1	—	1	—
B severe	13	3	4	2	1	2	—	—	—	—	—	—	—	—	1
B moderate	10	5	3	1	—	—	—	1	—	—	—	—	—	—	—
B mild	9	2	2	—	2	1	1	1	—	—	—	—	—	—	—
B total	32	10	9	3	3	3	1	2	—	—	—	—	—	—	1
Unclassified															
clinically severe	28	10	5	3	3	5	—	—	1	1	—	—	—	—	—
clinically mild	5	2	—	—	—	2	—	1	—	—	—	—	—	—	—
Clinically severe	104	39	25	16	5	9	2	3	1	3	—	—	—	—	1
Clinically moderate	34	18	5	8	1	—	—	1	—	—	—	—	—	1	—
Clinically mild	42	12	8	6	5	5	1	3	1	—	—	1	—	—	—
Total	180	69	38	30	11	14	3	7	2	3	—	1	—	1	1

moderate form) there had been altogether 13 haemophiliacs. The largest number of affected members, totally 14 was recorded in family 44 (haemophilia B, severe form). In respect of the total number of haemophilic members, there was no marked difference between families with haemophilia A and B, or those with the severe, moderate and mild form, respectively (*cf.* table V).

A report of coagulation studies on definite, probable and potential carriers has been given in an earlier paper (20). A close agreement was found between the genetically predicted proportion of actual carriers among potential carriers and the incidence of low levels of AHF or B factor among potential carriers.

Table VI lists the total number of known definite and probable carriers of haemophilia, as well as of known potential carriers with a 50 and 25 per cent genetic risk of being carriers. In families with clinically severe haemophilia, 19 definite or probable carriers, 81 potential carriers with a 50 per cent risk, and 61 potential carriers with a 25 per cent risk were born in the past three decades (1931—1960). One can therefore expect to find 75 carriers of fertile age in these families. The corresponding number of carriers is 29 for clinically moderate haemophilia and 54 for mild haemophilia.

Consequently it can be expected that both haemophiliacs and carriers will be born in these families during the next few decades.

Comments

Combined defects of coagulation factors in the same individual or in the same family have been reported by several

Table IV Number of descendants of carriers in Swedish haemophilic families

Haemophilia type	Descendants of carriers					Children of haemophiliacs	No. of families	No. of families with living haemophiliacs
	Haemophiliacs			Healthy sons	Daughters			
	Living	Dead	Total					
A severe	87	73	160	101	211	22	63	62
A moderate	37	18	55	24	55	23	24	23
A mild	59	27	86	55	99	90	28	28
A total	183	118	301	180	365	135	115	113
B severe	18	27	45	31	72	3	13	13
B moderate	14	7	21	4	15	10	10	10
B mild	20	12	32	17	34	28	9	8
B total	52	46	98	52	121	41	32	31
Unclassified								
clinically severe	8	71	79	53	111	1	28	3
clinically mild	10	9	19	15	23	6	5	5
Clinically severe	113	171	284	187	394	26	104	80
Clinically moderate	51	25	76	28	70	33	34	33
Clinically mild	89	48	137	87	156	124	42	41
Total	253	244	497	302	620	183	180	154

exception can be noted the south-east region contains 8 families (nos 14 44 59 60 118 134 153 and 173) with moderate or severe haemophilia B all of them originated from the county of Blekinge and its immediate vicinity A possible explanation is consanguinity between some of these families in earlier generations

Hereditary lines in Swedish haemophilic families

As stated earlier 23 families studied by Sköld (32) could not be classified by the type of coagulation defect, since they had no living male haemophiliac Altogether 19 of them had a possible heredity line, with a living female member of fertile age as far as could be ascertained, only four families had no potential female carrier Table IV shows the number of living and dead haemophiliacs, as well as the number of their sibs and children. It is seen that 154 of 180 families had a living haemophiliac Thus, only three families — in addition to the aforementioned 23 — had no living male haemophiliac.

The total number of living and dead haemophiliacs in the relevant families is listed in table V In 69 families, i. e 38 per cent, there was only one affected member Altogether 79 families, i. e 44 per cent, had two to four haemophiliacs. The remaining 32 families, i. e 18 per cent, each had five or more affected members. In family 27 (haemophilia A,

Potential carriers											
50 genetic risk Born years						25 % genetic risk Born years					
1900–1910	1911–1920	1921–1930	1931–1940	1941–1950	1951–1960	1900–1910	1911–1920	1921–1930	1931–1940	1941–1950	1951–1960
13	20	33	32	36	13	7	11	13	19	21	21
—	1	3	6	11	3	—	—	1	2	2	4
3	4	16	10	13	9	3	7	14	8	4	9
16	25	52	48	60	25	10	18	28	29	27	34

Table VII. Sporadic haemophilia reported in different series

Investigator	Country	Total no of families	Total no. of cases	Sporadic cases		Haemophilia A			Haemophilia B		
				No.	%	No. of families	Sporadic cases		No. of families	Sporadic cases	
							No.	%		No.	%
Aggeler et al. (1)	U.S.A.	34	—	11	32	21	7	33	13	4	31
Andreassen (2)	Denmark	63	—	20	32	—	—	—	—	—	—
Biggs & Macfarlane (4)	England	158	—	64	41	158	58	32	20	6	30
Breton et al. (7)	France	—	52	11	21	—	—	—	—	—	—
Cook (8)	Scotland	8	—	4	50	—	—	—	—	—	—
Davidson et al. (9)	U.S.A.	—	40	12	30	—	—	—	—	—	—
Deutsch (10)	Austria	47	—	20	43	35	15	43	12	5	42
Fonio (11)	Switzerland	81	—	35	43	—	—	—	—	—	—
Hartmann & Diamond (12)	U.S.A.	—	73	27	37	—	23	37	—	4	45
Hrodek & Herzmansky (14)	Czecho-Slovakia	—	90	29	31	—	24	33	—	5	28
Ikkala (15)	Finland	73	—	26	36	63	24	38	10	2	20
Nelson et al. (18)	Northern Ireland	—	39	22	55	—	21	60	—	1	25
den Ottolander (22)	Netherlands	79	—	22	28	71	20	28	8	2	25
Pavlovsky et al. (24)	Argentine	—	127	69	54	—	35	50	—	8	66
Pitney (25)	Australia	55	—	23	42	55	23	42	—	—	—
Rapaport et al (29)	U.S.A.	114	—	38	33	91	33	36	23	5	22
Sjølin (31)	Denmark	80	—	25	31	43	14	33	18	3	17
Sköld (32)	Sweden	60	—	20	33	—	—	—	—	—	—
Soulier (33)	France	—	81	37	46	—	20	51	—	7	41
Thomas et al. (36)	Canada	—	27	18	67	—	—	—	—	—	—
Present series	Sweden	173	—	46	27	115	34	30	32	6	19

Mild form only.
With only one known haemophiliac.
No combined deficiencies.
Seven families excluded owing to lacking family histories.

Table VI Living genetically established carriers

Haemophilia Clinical form	No. of families	Definite and probable carriers Born years					
		1900–1910	1911–1920	1921–1930	1931–1940	1941–1950	1951–1960
Severe	104	9	18	21	11	5	3
Moderate	34	3	10	3	7	4	6
Mild.	42	4	10	12	8	14	11
Total	180	16	38	36	26	23	20

authors. Sjolin (31) who recently published a survey of Danish haemophiliacs found 18 families (25 patients) with combined deficiencies of AHF and B factor and 7 families with different coagulation defects within the individual family Ikkala (15) in his study of 73 Finnish haemophilic families could not demonstrate any different coagulation defect in the 17 families with two or more haemophilic members tested Biggs & Macfarlane (4) in their study of *187 families*, observed no combined deficiencies of AHF and B factor in the 158 families investigated. In 13 families they found various combinations of haemostatic defects that made precise classification impossible. We found one family (no. 150) with moderate haemophilia B combined with a slightly low AHF level, but no case of different coagulation defects within the individual family Our findings are thus in agreement with those of Biggs & Macfarlane (4) and Ikkala (15) but opposed to that of Sjolin (31) This can be explained by Sjolin's methods of testing as pointed out in a previous paper (19)

Combined defects of B factor and factor VII have also been described (3 5 6 21 34 37) but no such combined deficiency was observed in our series.

Combined defects of AHF and factor V have been reported by several authors (10 16, 17 30) and our series contains a similar case with mild haemophilia A (family 109)

In Swedish haemophilic families, we found only two with combined coagulation defects these defects were thus rare in comparison with the Danish material presented by Sjolin (31)

We believe that it is of importance to *exclude the presence of any circulating* anticoagulant before making a diagnosis *of combined defects.* It follows that some of Sjolin's combined defects may in reality be a single defect plus an anticoagulant (*cf* 19)

Positive heredity in haemophilic families — by which is meant that the gene can be traced in at least two generations — has been reported from different parts of the world. As a rule, the number of sporadic cases of haemophilia has been given (table VII) It is apparent from this table that the incidence of sporadic cases, calculated on the total number of families, ranges from 50 to 27 per cent, the lowest figure being that in our series. The incidence in Scandinavian series ranges from 36 to 27 per cent (2 15 31 32) which is in good agreement with other investigations.

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The sporadic cases may be due to mutations in a parent generation or in an earlier generation (15, 33). The gene of haemophilia has, however, been known to skip five female generations, namely, in the Swiss Tenna family (13). Consequently, it is highly probable that many families in which the cases are denoted as sporadic do, in fact, have a positive heredity. This view is substantiated by the results of our studies on carriers of haemophilia A and B (20). Further investigations of carriers will presumably give the final answer to these questions.

The low incidence of sporadic haemophilia — *i. e.* without positive heredity in at least two generations — in our series is partly explained by the conclusions that can be drawn from the carrier investigations (20). It has been of invaluable help to have access to the family material collected by Sköld (32) in 1940—1943. In eight families (nos. 66, 79, 100, 110, 120, 133, 144 and 147) we have been able to trace members of "new" haemophilic families in his unpublished material. We could thus demonstrate a positive heredity despite the fact that the living haemophilic members were completely unaware of a family history of haemophilia.

Summary

Hereditary investigations have been made in 180 haemophilic families in Sweden. Altogether 173 families can be followed for three or more generations; a positive heredity is demonstrated in 127 of them, i. e. in 73 per cent. Coagulation studies in 147 families show that 115 of them have haemophilia A (AHF or factor VIII deficiency) and 32 have haemophilia B (B factor or factor IX deficiency). In 39 families, two or more haemophiliacs have been tested; all the affected members of the individual family are found to have the same type of haemophilia. A combined deficiency of B factor and a slightly low AHF level is recorded in all 5 tested members of one family. In another family low AHF in combination with a low factor V value is found in the only haemophiliac tested.

Altogether 26 of the 180 families have no living haemophiliac. Only four families have, as far as can be ascertained, no potential carrier of fertile age. It can be calculated from the pedigrees that there are altogether 75 carriers of severe haemophilia A and B of fertile age in Sweden. The corresponding number of carriers are respectively 29 and 54 for moderate and mild haemophilia A and B.

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From the Wilson Research Institut (Head Pentti I. Halonen, M. D.) Helsinki, Finland

The Precordial Isotope Dilution Curve in Mitral Stenosis and Correlations with the Clinical, Heart-catheterization and Roentgenologic Findings

By

Kari A. Pietilä and Juha Hakkila

Using the dye dilution method, studies have been carried out in mitral valvular disease, especially for differentiation between valvular stenosis and valvular insufficiency (1, 2). The dye dilution has generally been determined by means of a cuvette oximeter on arterial blood, or with an ear oximeter.

Radioactive indicators have made it possible to measure the dilution curve precordially without the necessity of making arterial punctures (3). In addition to giving information on the intracardial dilution of radioactivity in the right and left heart, the curve enables the pulmonary circulation time to be calculated from the time interval between the radioactivity peaks in the curve, and consequently also the pulmonary blood volume. Comparative studies have shown that this method gives reliable data on the cardiac output (4—10). Animal experiments suggest that the pulmonary blood volume calculated from the double-peaked precordial curve lies close to the measured blood volume of the lungs (11).

In mitral valvular disease the method has been used mainly for determination of the pulmonary blood volume (12, 13).

In the present work the circulation of radioactivity in the heart and lungs of patients with mitral stenosis, and of normal persons of the same age groups was studied by the precordial isotope dilution technique. The values calculated from the double-peaked isotope dilution curves were compared with the clinical severity of the disease, with values obtained by heart catheterization, and with the roentgenologic volume of the heart.

Method

For the precordial isotope dilution determinations, 30—40 μC of radioactive indicator I^{131}-albumin was injected into the basilic vein. The purity of the I^{131}-albumin

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Radioactive indicators have made it possible to measure the dilution curve precordially without the necessity of making arterial punctures (3) In addition to giving information on the intracardial dilution of radioactivity in the right and left heart, the curve enables the pulmonary circulation time to be calculated from the time interval between the radioactivity peaks in the curve, and consequently also the pulmonary blood volume. Comparative studies have shown that this method gives reliable data on the cardiac output (4—10) Animal experiments suggest that the pulmonary blood volume calculated from the double-peaked precordial curve lies close to the measured blood volume of the lungs (11)

In mitral valvular disease the method has been used mainly for determination of the pulmonary blood volume (12, 13)

In the present work the circulation of radioactivity in the heart and lungs of patients with mitral stenosis, and of normal persons of the same age groups, was studied by the precordial isotope dilution technique. The values calculated from the double-peaked isotope dilution curves were compared with the clinical severity of the disease with values obtained by heart catheterization, and with the roentgenologic volume of the heart.

Method

For the precordial isotope dilution determinations, 30—40 μC of radioactive indicator I^{131}-albumin was injected into the basilic vein. The purity of the I^{131}-albumin

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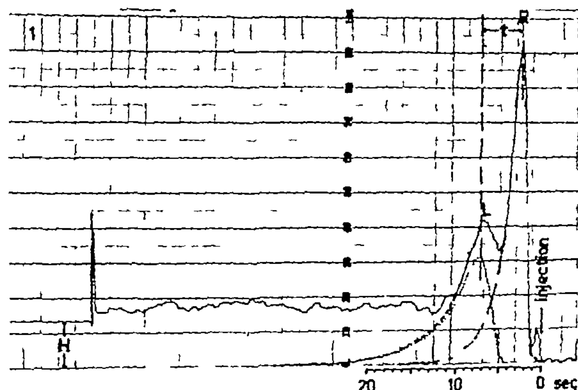


Fig 1 Precordial isotope dilution curve from a normal subject

R = right and L = left heart peak, H = height of the equilibrium level, t = peak-to-peak time, = extrapolated portions of the curve = curve drawn for the left side of heart.

Results from the curve Cardiac index (CI) 5.5 l/min., peak to-peak time (t) 4 8 sec., half-time of the second slope (h-t) 2 7 sec., pulmonary blood volume (PBV) 440 ml/m

was checked by dialysis and electrophoresis. The dilution curve was determined by means of a scintillation counter mounted in series with a ratemeter and a Varian G-10 recorder The counter was focused over the pulmonary arterial trunk and the left atrium, usually in the third left interspace parasternally The scintillation counter was shielded by a lead collimator having a cylindrical opening of 3 cm, diameter and 5 cm, depth, with a half value angle of 30° The examinations were made without sedation in the postprandial state, 2—4 hours after a meal, with the patient lying supine following 15—30 min. rest. During recording of the precordial curve the 0.5 sec time constant of the ratemeter was used After 10 min. the equilibrium level was determined with the time constant of 20 sec. and the venous blood sample was taken at the same time for measurement of the plasma volume.

The dilution curves obtained were double-peaked. The cardiac output the peak-to-peak time indicative of the pulmonary circulation time, and the pulmonary blood volume based on the latter were calculated from the curves as described earlier (14). When the peak from the left heart could not be determined with accuracy the curve for the left heart was drawn after extrapolation of the first peak and subtraction from the summation curve (fig 1) The cardiac output and the pulmonary blood volume were calculated per sq m of body surface. Additionally the half time of the second slope depicting the emptying rate of the left heart was measured by calculating the time in which the exponentially descending second slope declines to one-half of an arbitrary value.

The right heart catheterization of the patients with mitral stenosis was made in the usual

Table I. Results obtained by precordial dilution method and thorax X-ray in 40 cases of mitral stenosis

Case N	Age years	Class of N.Y.H. Ass.	Heart rhythm	Cardiac index l/min/m	Peak-to-peak time sec.	Half-time of second slope sec	Relative rtg heart volume ml/m	Pulmonary blood volume ml/m
1	38	II	sinus	3.3	8.6	3.9	530	475
2	37	II	sinus	3.7	7.1	4.2	480	442
3	17	II	sinus	4.6	5.2	3.3	350	398
4	55	II	sinus	3.2	9.9	7.8	6.0	520
5	47	II	sinus	2.9	7.1	5.5	590	542
6	48	II	sinus	3.8	9.7	4.4	600	581
7	46	III	sinus	1.7	13.4	13.3	840	358
8	28	III	sinus	2.0	10.1	13.6	730	328
9	37	II	sinus	2.0	14.5	9.8	640	477
10	41	III	sinus	2.0	14.6	10.1	700	481
11	50	II	sinus	2.0	13.3	10.2	630	452
12	24	II	sinus	2.1	12.0	7.3	690	250
13	24	II	sinus	2.8	7.5	6.5	560	353
14	35	II	sinus	3.5	8.9	4.4	540	525
15	45	II	sinus	3.6	8.9	5.9	520	536
16	33	II	sinus	2.6		11.5	600	
17	31	II	sinus	3.3	10.3	5.3	540	562
18	22	III	sinus	1.8		15.8	740	
19	21	II	sinus	2.0	11.2	10.6	70	374
20	40	III	sinus	2.4		12.1	620	
21	44	II	atrial	1.6	14.8	13.1	780	393
22	50	III	fibrillat.	1.7	11.5	13.1	750	329
23	58	III	fibrillat.	1.7	13.7	8.9	620	392
24	43	III	fibrillat.	1.8	14.1	9.2	820	415
25	43	III	fibrillat.	2.1	18.3	14.5	1,100	626
26	36	III	fibrillat.	2.1	11.8	9.2	870	414
27	37	II	fibrillat.	2.1	11.1	7.3	640	420
28	38	III	fibrillat.	2.3	15.7	8.9	680	600
29	35	III	fibrillat.	2.3	13.3	8.2	720	515
30	31	III	fibrillat.	2.3	8.5	7.9	520	350
31	44	II	fibrillat.	2.4	11.5	6.2	710	458
32	38	III	fibrillat.	2.0	14.2	10.5	710	498
33	51	III	fibrillat.	2.0	13.0	8.6	720	423
34	52	IV	fibrillat.	1.5	18.9	16.9	1,000	468
35	44	IV	fibrillat.	1.3	16.7	20.2	1,060	386
36	48	III	fibrillat.	2.4	11.7	9.2	850	462
37	43	III	fibrillat.	2.9	14.2	4.2	710	696
38	52	II	fibrillat.	2.5	10.1	7.4	630	421
39	37	IV	fibrillat.	1.8	18.2	17.0	950	330
40	57	III	fibrillat.	1.7	16.3	18.0	880	456

manner. The dye dilution curves were determined by the ear oximeter after injection of Evans' blue into the pulmonary artery.

The roentgenologic volume of the heart was calculated from postero-anterior and lateral chest films made from the standing patient at focus distance of 1.5 metre (15). The relative heart volume was calculated per sq. m of body surface.

The intervals between the heart catheterisation, X-ray exposure and precordial dilution determination varied from 1 to 7 days.

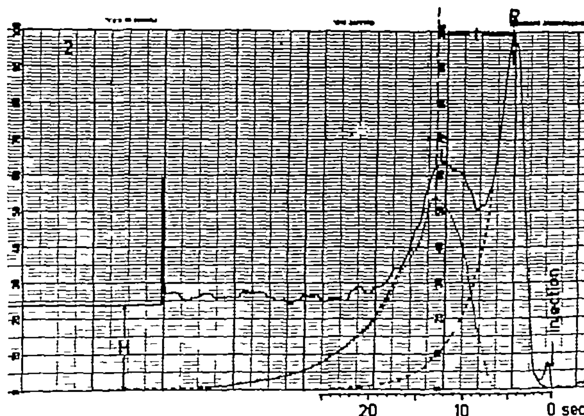


Fig 2. Precordial curve from a patient with mild mitral stenosis. Normal cardiac index and slight prolongation of the peak-to-peak time.
Results as calculated from the curve (symbols as in fig 1) CI 3.3 l/min., t 8.6 sec., h-t 3.9 sec., PBV 475 ml/m

Material

The series consisted of 40 patients with mitral stenosis. The diagnosis was based on clinical examination and chest films. Right heart catheterization was carried out on 38 patients, it having failed in 2 cases. Commissurotomy was performed later in 33 cases. The operated cases included the 2 patients whose heart catheterization had been unsuccessful. In 5 cases commissurotomy was not recommended because of the mild degree of stenosis. Two of the examined patients declined an operation.

The mean age of the patients, 25 of whom were women, was 40 years (range 21—55). The class of physical performance was determined according to the criteria of the New York Heart Association (16). There were 19 patients in class II 18 patients in class III and 3 patients in class IV. At the time of examination 20 patients had atrial fibrillation.

The control series consisted of 30 subjects with no evidence of cardiopulmonary disease mean age 41 years (range 18—64). Seven of the controls were women.

Results

The precordial isotope dilution curves of 40 patients with mitral stenosis and of 30 normal subjects were analysed. A double peaked curve was obtained in all cases. In 3 patients with mitral stenosis the peak to-peak time could not be determined with sufficient accuracy (< 0.3 sec.). These cases were therefore excluded from calculations of the peak to-peak time and the pulmonary blood volume.

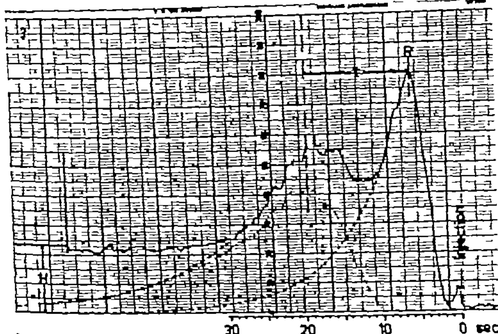


Fig. 3. Precordial curv from a patient with mitral stenosis and low cardiac index. Marked prolongation of the peak-to-peak time.
Results from the curv (symbols as in fig. 1) CI 2 3 l/min., 13.3 sec h-t 8.2 sec., PBV 515 ml/m²

SHAPE OF DILUTION CURVE

In mild cases of mitral stenosis the ilution curve differed but slightly from he curve for a normal subject (figs. 1 nd 2) With decrease of the physical erformance the peak-to-peak time was rolonged and the slope of the second peak descended more slowly than in the normal subject curve (fig 3) In the most severe cases (classes III and IV) the additional finding was made that both peaks were broad and the second peak was relatively high in comparison with the first peak (fig 4)

VALU CA LATED FROM THE DILUTION ES

Cardiac inde Fig 5 shows the variations the cardiac index in normal subjects and in patients with mitral stenosis. The mean cardiac index in normal subjects was 4 4 l/min. Patients with mitral stenosis had in class II a mean cardiac index of 2.8 l/min. Values corresponding to the cardiac index of normal subjects were seen only in patients with sinus rhythm in this class. There was a distinct difference in regard to the cardiac index between patients with sinus rhythm (mean 3 0 l/min.) and those with trial fibrillation (mean 2.2 l/min.) in class II In class III the mean cardiac index was 2.1 l/min. and no difference was seen between patients with sinus rhythm and those with atrial fibrillation. In class IV the mean cardiac index was 1.5 l/min.

It was observed that n patients w th

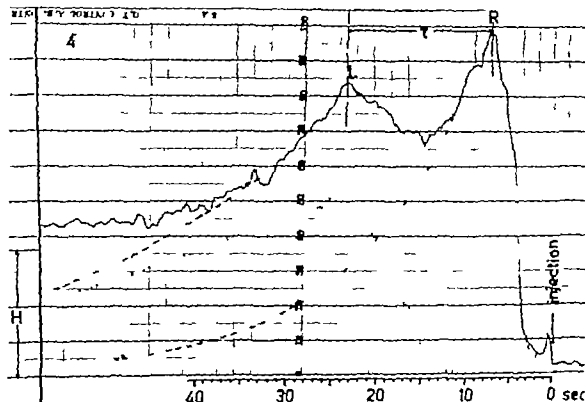


Fig 4 Case of severe mitral stenosis with low cardiac index. Highly prolonged peak-to-peak time Results from the curve (symbols as in fig 1) CI 1 7 l/min. t 16 3 sec., h-t 18.0 sec., PBV 456 ml/m²

a low cardiac output the time required for the primary circulation may be as long as 20 sec. In such cases the re circulation, which in the precordial curve *usually is not clearly visible* perhaps reaches the field of the scintillator before the end of the primary circulation, thus decreasing the cardiac output reading For this reason, I¹³¹-albumin and Evan's blue dye were injected simultaneously and the time interval between the primary circulation and the re-circulation was determined from both the precordial curve and the curve recorded by the ear oximeter Comparison of these curves showed that even in cases of low output the recirculation enters the field of the scintillator after the primary circulation in the precordial curve.

Pulmonary circulation time The mean peak to-peak time in normal subjects was 6 0 sec. In patients with mitral stenosis the peak to-peak time was prolonged with increasing impairment of the physical performance (fig 5 b) In class II the patients with sinus rhythm had a mean peak to-peak time of 9 6 sec. and the patients with atrial fibrillation 11 9 sec In class III the mean peak to-peak time was 13 4 sec. and in class IV 17 9 sec Peak to-peak times that may be regarded as normal were encountered in class II in 7 of 14 patients with sinus rhythm and in class III in 1 of 13 patients with atrial fibrillation

Pulmonary blood volume Normal subjects had a mean pulmonary blood volume of 423 ± 16 ml/m² In patients with mitral stenosis the mean was 454 ± 16 ml/m² There was no statistically significant difference between the means (P = fig 5 c) The pulmonary blood volume

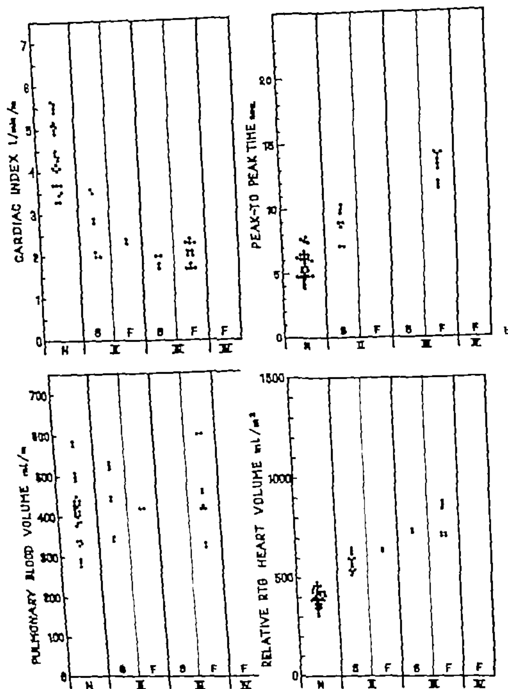


Fig. 5. Comparison of results for normal subjects and for patients with mitral stenosis grouped into classes II–IV (N.Y. Heart Ass.)
(a–d). N = normal subjects, S = patients with mitral stenosis and sinus rhythm, F = patients with mitral stenosis and atrial fibrillation.

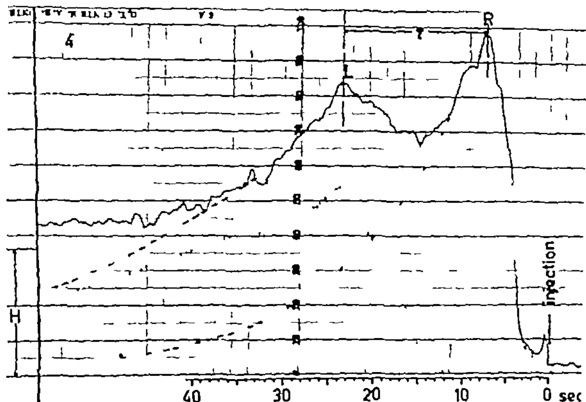


Fig 4 Case of severe mitral stenosis with low cardiac index. Highly prolonged peak-to-peak time. Results from the curve (symbols as in fig. 1) CI 1 7 l/min., t 16 5 sec., h-t 18.0 sec., PBV 456 ml/m

a low cardiac output the time required for the primary circulation may be as long as 20 sec In such cases the re circulation, which in the precordial curve usually is not clearly visible, perhaps reaches the field of the scintillator before the end of the primary circulation, thus decreasing the cardiac output read ing For this reason I[131] albumin and Evan s blue dye were injected simul taneously and the time interval between the primary circulation and the re-circulation was determined from both the precordial curve and the curve recorded by the ear oximeter Comparison of these curves showed that even in cases of low output the recirculation enters the field of the scintillator after the primary circulation in the precordial curve.

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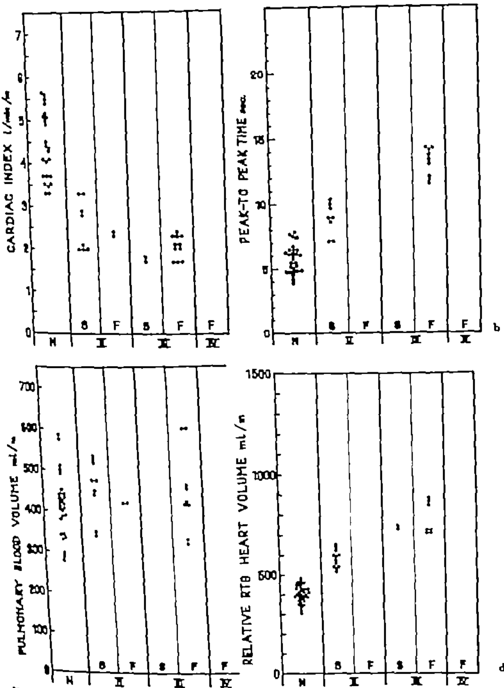


Fig. 3. Comparison of results for normal subjects and for patients with mitral stenosis grouped into classes II–IV (N.Y. Health Ass.).
a–d. N = normal subjects, S = patients with mitral stenosis and sinus rhythm, F = patients with mitral stenosis and atrial fibrillation.

Table II Comparison of correlations in mitral stenosis between the results obtained by precordial dilution method, roentgenologic heart volume measurement and right heart catheterization results

	Cardiac index	Half time of second slope	Peak to-peak time	Relative rtg heart volume	Pulmonary blood volume	Total pulmonary resistance	Pulmonary vascular resistance	Pulm. art. mean pressure	Pulm. wedge mean pressure
Cardiac index		++ −0.85	++ −0.80	++ −0.73	—	+ −0.51	+ −0.46	++ −0.59	++ −0.45
Half-time of second slope	++ −0.85		++ +0.76	++ +0.77	—	++ +0.58	+ +0.46	+ +0.34	—
Peak-to-peak time	++ −0.80	++ +0.76		++ +0.83	—	—	(+) +0.45	—	—
Relative rtg heart volume	++ −0.73	++ +0.77	++ +0.83		—	—	(+) +0.41	—	—
Pulmonary blood volume	—	—	—	—		—	—	—	—

++ = highly significant, + = significant and (+) = almost significant correlation, — = no correlation. = semilogarithmic correlation, = logarithmic correlation, = correlation observed only in patients with sinus rhythm. Minus or plus value = correlation coefficient r

averaged 16 0 per cent and 16 8 per cent of the total blood volume in the normal subjects and in patients with mitral stenosis, respectively Between the various classes there was no essential difference in the pulmonary blood volume.

ROENTGENOLOGIC HEART VOLUME

The mean relative heart volume was 403 ml/m² in normal subjects. In patients with mitral stenosis the heart volume was found to increase with increasing impairment of the physical performance (fig 5 d) In class II the mean relative heart volume was 593 ml/m² in class III 757 ml/m² and in class IV 1 005 ml/m² Comparison between normal subjects and patients with mitral stenosis in the different classes revealed changes in the heart volume similar to the changes in the cardiac index and peak to-peak time.

RESULTS OF CORRELATION STUDY

Studies of correlation of the values calculated from the precordial curves, the results of catheterization and the roentgenologic heart volume are shown in table II No correlation was seen with the pulmonary blood volume per square metre. Only the cardiac index showed a significant correlation with the other calculated values. The values obtained from the precordial dilution curves had significant correlations with each other and with the relative heart volume (figs. 6 and 7) Among the values calculated on the basis of heart catheterization the pulmonary vascular resistance showed the best correlation

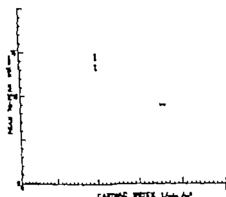


Fig. 6. Graphic comparison of cardiac index and peak-to-peak time of patients with mitral stenosis.

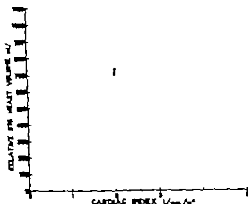


Fig 7 Graphic comparison of cardiac index and relative roentgenologic heart olume of patients with mitral stenosis.

Comments

The results of studies of normal subjects based on the precordial isotope dilution technique are not always directly comparable because of differences in the series studied, experimental conditions and methods. Thus, using the precordial technique, cardiac index values varying in the range 3.3—4 7 l/min./m have been obtained in normal subjects (4 12 15, 17) Using the same type of method as that in the present study the mean cardiac index in 41 normal subjects in the postprandial state was 4 7 l/min./m (in basal state 4.0 l/min./m²) (12) which is close to the value of 4.3 l/min./m obtained in the present study In an earlier study in this laboratory using simultaneous precordial and arterial dilution methods, the mean cardiac outputs in thirty-four determinations were 7 78 l/min. and 7.58 l/min., respectively (10)

With the peak-to-peak method of calculation in the precordial technique the mean pulmonary circulation time in 24 normal subjects was found to be 5.8 sec. (18) the value was 6 0 sec in the present work. The so-called mean pulmonary circulation time gives a considerably longer pulmonary circulation time than the peak to-peak time as is indicated by the mean values of 11 4 (12) and 11 1 sec. (13) in normal subjects. It has been stated that the pulmonary circulation times calculated by these methods are influenced by the rate of emptying of the right and left hearts (19)

The pulmonary blood volume value is naturally influenced also by the method of calculation of the pulmonary circulation time. Using the mean pulmonary circulation time, the mean pulmonary blood volume in normal subjects in the postprandial state was 610—641 ml/m (12, 13) whereas the mean pulmonary blood volume in the present study was 431 ml/m When the pulmonary circulation time was calculated on the basis of successive dye injections into the left atrium and the pulmonary artery a mean pulmonary blood volume of 365 ml/m was obtained in patients with heart disease, predominantly mitral stenosis (20)

The cardiac index values obtained in the present study for patients with mitral stenosis were somewhat lower when compared with the results of previous studies using Fick's method and the same classification of physical performance (21 22) In the present study the mean cardiac output values obtained by the precordial technique were nearly equal to those obtained by Fick's method.

The pulmonary blood volume in mitral stenosis was within normal limits. Similar observations have been made in several previous investigations with regard to the so-called central blood volume using the arterial dye sampling method (23 24) and the pulmonary blood volume using the precordial isotope dilution method (12 13) Perhaps in patients with mitral stenosis, especially in the presence of constriction of the peripheral branches of the pulmonary artery in the lower portions of the lungs, a part of the pulmonary circulation is delayed and is not recorded during the primary circulation, thus giving an erroneous pulmonary blood volume determination (25)

As in earlier published results of heart catheterization of patients with mitral stenosis (22, 26) the present correlation study indicated that the cardiac output has a significant correlation with the mean pulmonary arterial pressure, the mean pulmonary wedged pressure and the pulmonary vascular resistances. The negative correlation observed between the cardiac index and the pulmonary circulation times is in accordance with the results obtained by the precordial technique in patients with mitral disease (12) A similar observation in patients with mitral stenosis has been made by the arterial sampling method (27) In the latter study also a negative correlation between the cardiac index and cardiothoracic ratio was observed. In addition to retardation of the pulmonary circulation time due to the reduced cardiac output, there evidently also are other factors that influence the pulmonary circulation time when this is measured by the precordial technique This is suggested by the greater prolongation of the pulmonary circulation time relative to the change in the cardiac index A contributing factor may be the increased amount of residual blood in the heart with increase of heart size, which causes a delay of the injected indicator in the right side of the heart. This opinion is supported by the correlation seen in the present study between the pulmonary circulation time and the relative heart volume. In angiocardiographic studies a prolonged flow of opacified blood into the left heart was observed in cases with a longer right heart opacification time (28) In same studies the intracardiac circulation time from initial opacification of the right atrium to initial opacification of the ascending aorta in persons with normal hearts did not exceed 6 3 seconds

On basis of the correlation between the cardiac index and the roentgenologic heart volume, it seems possible that the rontgenologic relative size of the heart can be employed in clinical work for evaluation of the cardiac index in patients with mitral stenosis.

Summary

Double-peak precordial isotope dilution curves of 40 patients with mitral stenosis and of 30 normal subjects were analysed. From the curves were determined the cardiac index, peak to-peak time pulmonary blood volume based on the peak to-peak time, and half time of the second slope

The mean cardiac index in normal subjects was 4.4 l/min./m and in patients with mitral stenosis in classes II—IV 2.8, 2.1 and 1.5 l/min./m respectively.

With increasing severity of the mitral stenosis the peak-to-peak time of the dilution curve was prolonged, almost threefold in patients in class IV (N. Y. Health Ass.) compared with normal.

The mean pulmonary blood volume in normal subjects was 425 ml/m and in patients with mitral stenosis 454 ml/m. There was no statistically significant difference between the means.

The results of correlation studies between the values obtained from the dilution curves, right heart catheterization and roentgenologic heart volume are presented.

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Contraction of Salmonella Gastroenteritis Following Previous Operation on the Stomach

By

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In recent years we have now and then observed patients, suffering from Salmonella gastroenteritis, who had previously been subjected to gastric surgery and we have frequently discussed whether an association of these conditions exists or not.

Very little attention has been paid to this subject in the literature. Waddell and Kunz (1956) observed nine cases of enteric infections with Salmonella and a previous history of major gastric surgery a group accounting for no less than 26 per cent of Salmonella infections in adults admitted to the Massachusetts General Hospital, Boston, in 1954 and 1955 These authors suggest that patients who have previously undergone operations on the stomach run a considerably higher risk of contracting Salmonella gastroenteritis than the average patient with a normal gastrointestinal system.

Although the statement of Waddell and Kunz is based on a small material (34 patients) it is nevertheless an interesting one, particulary because the association proposed might be explained by changes in the physiology of the alimentary tract following operation. An attempt to elucidate the problem further is presented in the present paper The incidence of patients who have had previous gastric surgery among adult cases of salmonellosis from two Swedish hospitals for infectious diseases is reported. A group of patients with other diseases, infectious or not, serves as a material for comparison.

Material

The material consists of all patients above 20 years of age with salmonellosis, admitted to the Hospitals for Infectious Diseases at Uppsala and Örebro, Sweden, during the years 1954—58. The number of cases from Uppsala is 159 from Örebro 118, altogether 277 cases. The great majority of these patients had Salmonella gastroenteritis, and the organisms were cultured from the stools. A few patients had paratyphoid fever in these cases the organisms were frequently recovered from the blood or the urine Many were bacillary carriers only traced by epidemiologic investigations of the environment of those affected. The different types of Salmonella organisms isolated are listed in table I.

Submitted for publication December 14 1961

Table I Types of Salmonella organisms isolated from the cases

Type	No. of cases
S. typhimurium	143
S. enteritidis	61
S. montevideo	20
S. paratyphi B	13
S. oranienburg	11
S. cholerae suis	5
S. newport	4
S. carrau, S. infantis (each)	3
S. cubana, S. bareilly	
S. muenchen, S. javiana (each)	2
S. napoli, S. stanley	
S. chester S. typhi (each)	1
No type	3
Total	278

Infection with both S typhi and S. cubana in one case.

Table II Various types of diseases constituting the control material

Type of disease	No. of cases
Acute infectious diseases	209
Respiratory diseases	85
Gastrointestinal diseases other than gastroenteritis	52
Neurologic diseases	21
Malignant, metabolic and systemic diseases	20
Urinary tract diseases	12
Circulatory diseases	10
Skin diseases	9
Various diseases	18
Total	436

Table III Incidence of patients who had undergone gastric surgery in the salmonellosis and control materials

	No.	Cases with gastric surgery	
		No.	
Salmonellosis group			
Cases with symptoms	195	15	7.7
Bacillary carriers	82	1	1.2
Total	277	16	5.8
Control group	436	9	1.8

Table IV Incidence of patients who had undergone gastric surgery in the salmonellosis and control materials from two hospitals

	No.	Cases with gastric surgery	
		No.	%
Salmonellosis group (Uppsala)			
Cases with symptoms	102	9	8.8
Bacillary carriers	57	1	1.8
Total	159	10	6.3
Control group (Uppsala)	318	6	1.9
Salmonellosis group (Örebro)			
Cases with symptoms	93	6	6.5
Bacillary carriers	25	0	—
Total	118	6	5.1
Control group (Örebro)	118	2	1.7

The material for comparison in the following designated the control material, consists of patients with various diagnoses. Cases of gastroenteritis of other causes than Salmonella infection have been excluded, because it is conceivable that persons with a previous operation on the stomach might have a tendency to contract such enteric infections as well. For the same reason cases of infectious hepatitis and poliomyelitis have also been excluded from the control material. For each patient with salmonellosis the next (Örebro) or the next two (Uppsala) patients in the hospital file of the same age group have been taken as controls. Consequently the control material consists of 436 patients. The various types of diseases of the patients in the control material are listed in table II.

Table V Incidence of patients who had undergone gastric surgery in different age groups

Age (years)	Salmonellosis group			Control group		
	No.	Cases with gastric surgery		No.	Cases with gastric surgery	
		No.	%		No.	%
20–39	139	3	2.2	230	1	0.4
40–59	90	7	7.8	138	5	3.6
> 60	48	6	12.5	68	2	2.9

Table VI Incidence of male and female patients with previous gastric surgery

	Males			Females		
	No.	Cases with gastric surgery		No.	Cases with gastric surgery	
		No.	%		No.	
Salmonellosis group	113	15	13.3	164	1	0.6
Control group	209	7	3.4	227	1	0.4

Results

The number of patients who had undergone gastric surgery in the salmonellosis and control groups from the two hospitals is recorded in table III. It is seen from the table that such patients are 3.2 times as frequent in the salmonellosis group as in the control group or if only cases with symptoms are considered, 4.3 times as frequent.

The incidence is approximately the same in the two hospitals, as is seen from table IV. Patients who had had operations on the stomach occur 3.5 times as frequently in the salmonellosis material from Uppsala as in the control material from the same hospital, or if only cases with symptoms are taken into account, are 4.6 times as frequent. The corresponding figures from Örebro are 3.0 and 3.8 respectively.

In table V the patients are divided into age groups. As expected, the incidence of gastrectomised patients increases with increasing age. The incidence is from — to 5.5 times higher in the different age groups of the salmonellosis material than in the corresponding age groups of the control material.

The results presented have thus revealed a higher incidence of persons with previous operations on the stomach among patients with salmonellosis than among patients with other diseases. This association appeared in two hospitals from two different geographical areas and it was found to occur independently of age. Further the same relationship was almost constantly observed when the material was divided into small subgroups according to age and year of contracting the illness. Statistical analysis revealed that it is unlikely that the association observed could have occurred by chance.

As expected, males predominate among the patients who had been operated on previously. Of the 16 patients with previous gastric surgery in the salmonellosis group, 15 are males and only one is female. Of the eight patients in the control group seven are males and one is female. In fact the difference in incidence of persons with previous operations on the stomach between the two groups of patients is only observed among males, as is seen from table VI.

All the 16 patients of the salmonellosis group had been operated on because of gastric or duodenal ulcer. Six patients of the control group had undergone

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Table VII Some clinical data for the patients of the salmonellosis group who had undergone gastric surgery

Case no.	Age at present illness (yrs.)	Time from op. to present illness (yrs.)	Diagnosis at op.	Type of operation	Type of salmonellosis	Remarks
1	72	31	G U	Gastrostomy + post. G E.	Gastroenteritis (S. typhimurium)	Severe symptoms. No other cases in environment
2	60	19	G U	Gastrect. B I 1/3 removed	Gastroenteritis (S. typhimurium)	No other cases in environment
3	81	18	D U	Gastrect. Polya. 2/3 removed	Enteritis (S. muenchen)	Sister of pat. the same disease
4	80	16	D U	Gastrostomy + post. G E.	Gastroenteritis (S. typhimurium)	Severe symptoms. Wife of pat. the same disease
5	59	11	G U	Gastrect. B II + post. G E. "High resection"	Gastroenteritis (S. typhimurium)	Two sons the same disease
6	76	5	G U	Gastrect. B II + ant. G E. 2/3 removed	Enteritis (S. montevideo)	Secondary cases in household
7	44	5	D U	Gastrect. B I	Gastroenteritis (S. typhimurium)	No other cases in environment
8	32	5	D U	Gastrect. B II + ant. G E. 2/3 removed	Carrier (S. typhimurium)	Husband to pat. no. 16
9	40	4	G U	Gastrect. B II + ant. G E. 3/4 removed	Gastroenteritis (S. typhimurium)	No other cases in environment
10	46	3	G U	Gastrect. B II + post. G E.	Enteritis (S. typhimurium)	No other cases in environment
11	51	3	G U	Gastrect. B II + post. G E. "Moderately high resection"	Gastroenteritis (S. typhimurium)	Wife of pat. the same disease
12	9	3	D U	Gastrect. B II + ant. G E. "High resection"	Gastroenteritis (S. typhimurium)	No other cases in environment
13	41	3	D U	Gastrect. B II + ant. G E. 2/3 removed	Gastroenteritis (S. carrau)	Source of inf. bacillary carrier. Pat. the only one to be infected
14	67	2	G U	Gastrect. B I "High resection	Gastroenteritis (S. typhimurium)	Wife of pat. the same disease
15	51	7/12	G U	Gastrect. B II + ant. G E. 2/3 removed	Gastroenteritis (S. montevideo)	Severe symptoms. Friend bacillary carrier after eating the same meal
16	36	3/12	G U	Gastrect. B II + ant. G. E. 2/3 removed	Gastroenteritis (S. typhimurium)	Severe symptoms. Husband and daughter carriers

G U = gastric ulcer D. U = duodenal ulcer G E. = gastroenterostomy

Table VIII. Some clinical data for the patients of the control group who had undergone gastric surgery

Case no.	Age at present illness (years)	Time from op. to present illness (years)	Diagnosis at op.	Type of operation	Present illness
1	35	5	Gastric polyposis + Achylia	Gastrect. B II	Paralysis agitans
2	55	4	Duodenal ulcer	Gastrect. B I 2/3 removed	Acute pyelonephritis
3	64	3	Gastric carcinoma	Gastrect. B II + ant. G. E. 2/3 removed	Cardiosclerosis
4	68	2	Duodenal ulcer	Gastrect. B II + ant. G. E. "Moderately high resection	Labyrinthitis
5	41	2	Duodenal ulcer	Gastrect. B II + ant. G. E. 2/3 removed	Aseptic meningitis
6	36	1	Gastric ulcer	Gastrect. B II + G. E. "Semi-high resection	Chickenpox
7	35	8/12	Gastric ulcer	Gastrect. B II + post. G. E. 4/5 removed	Bronchopneumonia
8	49	6/12	Gastric ulcer	Gastrect. B II + ant. G. E. 2/3 removed	Staphylococcal septicemia

surgery for the same reason one was operated on because of cancer of the stomach and one because of gastric polyposis. Some data concerning the patients operated on are recorded in tables VII and VIII

It is seen from the tables that the time interval between operation and contraction of Salmonella infection is from three months to 31 years, and the time interval between operation and present illness for the patients of the control group is from six months to five years. In most cases a high resection was performed. Gastroenterostomy only was carried out on two patients in the Salmonella material (table VII cases 1 and 4). In seven instances (table VII cases 1 2, 7 9 10 12 and 13) no other person in the environment contracted salmonellosis, despite sometimes eating the same food as the patient. In at least two instances (table VII cases 6 and 16) the patient was regarded as the focus of infection for members of the family In four instances (table VII cases 3, 5 11 and 14) it was not possible to judge the epidemiologic relationship between the members of the household. In two instances (table VII cases 4 and 15) other persons contracted salmonellosis after eating the same meal. In the latter cases it is of some interest to note that the patient with a previous operation on the stomach developed considerably more severe symptoms than the other patient with a normal alimentary tract

Discussion

As has been pointed out, the observed difference in incidence of patients who have undergone major gastric surgery between a group of patients hospitalized because of salmonellosis and a group of patients hospitalized because of other diseases could not have occurred by chance. However difficulties in interpretation of the relationship observed arise from possible differences in behaviour between different groups of patients, as it always does in investigations of this type on hospital populations.

Firstly it is quite likely that patients with a previous operation on the stomach are particulary inclined to seek medical attention when contracting any *illness*, because of their previous experiences or because of their type of personality or because of anxiousness among relatives. The doctor consulted might also be particularly prone to refer these patients to the hospital. However this tendency would seem to wane with the years after the operation and as has been mentioned, in many instances several years had passed between the operation and the current illness. Further if such a disposition exists it should have an effect on the control group as well. In fact the incidence of gastrectomised patients in the control material seems rather high (1 8 %) although reliable figures for the true incidence within the population are difficult to obtain

A few of the patients with Salmonella gastroenteritis, who had undergone major operations on the stomach, developed particulary serious symptoms. Isolated reports of a similar course are found in the literature (Förster and Leopold 1953 Abel 1955 Aufdermaur 1957) However it seems not to be proved that such a course of events is typical of salmonellosis in this type of patient Were it so it would certainly add to the proneness to seek a doctor's help

An important question is whether the hospitalized patients with salmonellosis represent only a small part of all persons with salmonellosis within the hospital area or a high percentage of these cases. Certainly many cases of Salmonella gastroenteritis exhibit only mild symptoms of short duration and the true nature of the disease will never be revealed. All persons with a positive fecal culture are compulsorily admitted according to Swedish law Thus the likelihood that cases of salmonellosis will be traced and hospitalized depends on several factors the severity of the symptoms, the willingness to consult the doctor or the nurse, and the readiness of the medical personnel to recommend taking a fecal specimen for bacterial culture. It may however be considered reasonable to suppose that a high percentage of the salmonellosis cases will be admitted, and the material of hospitalized patients is thus adequately representative.

Due to intolerance of various kinds of foodstuffs, many gastrectomised patients have to modify their eating habits. This might lead to a preponderance of a kind of food which under certain circumstances is known to be proportionately more dangerous with regard to contamination than other kinds, e g meat. I have not found it possible to *estimate* to what degree such a change in diet may increase the risk of contracting Salmonella *infection* It should be remembered, however that many patients find some *time* after the operation that they are able to eat regular foods without discomfort (Meurling 1953) Thus the kinds

of food these patients eat do not necessarily differ from those of the family or the environment.

The most reasonable explanation of the frequent occurrence of gastrectomised patients among cases of salmonellosis seems to be, as was pointed out by Waddell and Kunz that these patients probably run a higher risk of contracting Salmonella infection than patients with normal gastrointestinal systems.

The considerable changes in the physiology of the gastrointestinal tract following operation can readily explain the presumed greater risk of contracting salmonellosis. The changes following gastrectomy are summarized by Meurling as follows. "The capacity of the stomach is greatly decreased by the removal of about 2/3 of it, and the gastric remnant empties much quicker than the normal stomach. The gastric stage of digestion becomes deficient, in part due to the achylia common after gastrectomy The achylia also allows an abnormal bacterial flora to establish itself in the upper small intestine and stomach."

It is easily understood that the changes described favour an infection. The normal gastric juice has a considerable power of disinfection (Garrod 1937) and most organisms are destroyed during the stay of the swallowed food in the stomach. In fact, it is generally considered that a large dose of Salmonella organisms has to be consumed to bring about symptoms of disease in the normal man (Harries and Mitman 1951 Olm 1956) although relatively low doses of some Salmonella species were occasionally found to produce disease in feeding experiments on human beings (McCullough and Eisele 1951) In the gastrectomised patient the common achylia prevents sterilization, and the microbes reach the intestines rapidly It appears likely that a relatively low dose of organisms is sufficient to produce symptoms, particularly in the gastrectomised patient with achylia, a dose which will be completely destroyed in the stomach with a normal acidity and a normal mechanism of emptying These circumstances should explain the association of previous major gastric surgery and salmonellosis. However during smaller or larger outbreaks of Salmonella food poisoning when the doses of bacteria swallowed are large, the association will probably not appear

Summary

The occurrence of cases with previous major operations on the stomach within a group of adult patients with salmonellosis has been examined, compared with the frequency of the same type of case within a control group of patients hospitalized for other reasons. It has been revealed, from two different hospitals in Sweden, that patients who have undergone gastric surgery previously appear about four times as frequently in the salmonellosis material as in the control material. This association appears to be independent of age but is only found among men.

The explanation of the association observed is probably to be found in the changes in the physiology of the alimentary tract following surgery changes which enhance the chances of contracting Salmonella gastroenteritis following ingestion of infected food.

Acknowledgement

I am indebted to Jan Barr M D for allowing me to obtain material from the Hospital for Infectious Diseases, Orebro

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